

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**204820Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## Secondary Pharmacology and Toxicology Review for NDA 204-820

TO: NDA 204-820 (Hikma Pharmaceuticals, LLC)

FROM: Marcie Wood, Ph.D.  
Supervisory Pharmacologist  
Division of Pulmonary, Allergy, and Rheumatology Drug Products

DATE: July 8, 2013

Overview: I concur with the recommendation of Dr. L. Steven Leshin (detailed in a nonclinical review dated July 1, 2013) that Mitigare (0.6 mg colchicine capsules) should be approved from a nonclinical perspective.

Mitigare is a new formulation of colchicine (0.6 mg colchicine capsules) indicated for the prophylaxis of gout flares. Colchicine has a long history of clinical use for the treatment of gout. The applicant has submitted this application in response to an FDA drug safety initiative to bring marketed, unapproved drugs under NDA. No new nonclinical studies were submitted for review; rather, the applicant is relying on published nonclinical literature in support of this 505(b)(2) application.

Toxicology: Historical information provided by the applicant from published animal and clinical studies indicated similar toxicities. In general, the acute toxic signs in animals (rats, dogs, rabbits, cats) with short-term colchicine administration are gastrointestinal tract-related. With increasing doses these signs become more severe, and there is a loss of body tone, abnormal gait and hindlimb paralysis, and wasting atrophy, ascites, and eventually death. However, the published nonclinical literature contained inadequate long-term toxicology studies. Almost all the studies were conducted prior to GLP regulations and lack much of the information now routinely assessed, such as clinical pathology and histopathology findings. A direct NOAEL comparison could not be conducted, since nonclinical studies were not conducted with the goal of identifying a NOAEL. Rather, the studies were conducted to identify toxicities or underlying biological mechanisms of colchicine action. Conduct of chronic, GLP-compliant animal toxicity studies with colchicine, though, would not likely produce any additional useful information, as colchicine toxicity is known to be similar across all species (including humans) and there is already a long clinical history of colchicine use for the treatment of gout and understanding of clinical colchicine toxicity.

Genotoxicity: Colchicine was negative for mutagenicity in the bacterial reverse mutation assay. In a chromosomal aberration assay in cultured human white blood cells, colchicine treatment resulted in the formation of micronuclei. However, published studies demonstrated these micronuclei were formed by mitotic nondisjunction without structural DNA changes. Therefore, colchicine is also not considered clastogenic.

**Carcinogenicity:** There are no adequate studies of colchicine's carcinogenic potential in standard rodent bioassays. Due to the long history of clinical experience and age of the patient population, carcinogenicity studies were not requested for this application. However, the theoretical risk for tumorigenesis due to colchicine's aneugenic properties will be indicated in the package insert.

**Reproductive and Developmental Toxicology:** The nonclinical literature indicates that colchicine has detrimental effects on reproduction and development, including fertility, early embryonic development, and organogenesis. The effects are species and dose dependent, and the timing of exposure is also critical to reproductive outcome. The risk for reduced fertility and embryofetal harm, as indicated published animal studies, should be conveyed in product labeling. However, it is noted that clinical epidemiology studies in patients with familial Mediterranean fever found that colchicine therapy was compatible with normal reproduction and pregnancy in the therapeutic dose range. It is not known if a similar degree of safety is evident in patients taking colchicine for gout, for which the therapeutic dose is usually less than for familial Mediterranean fever. However, the patient population with gout is typically beyond the child-bearing age.

**Labeling:** Dr. Leshin has recommended labeling edits for Sections 8.1 (Pregnancy), 12.1 (Mechanism of Action), and 13.1 (Carcinogenesis, Mutagenesis, and Impairment of Fertility) based on nonclinical information available in published literature. See Dr. Leshin's review for complete product labeling details. I agree with the proposed labeling edits. Labeling negotiations with the applicant are currently ongoing.

Despite lack of a complete nonclinical development program by modern standards, publically available nonclinical data that parallel what is already known about clinical colchicine toxicity appear to support approval of Mitigare from a nonclinical perspective. Nonclinical knowledge gaps (e.g. carcinogenicity) can be addressed with labeling. Therefore, there are currently no outstanding Pharmacology and Toxicology issues for this product.

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/s/  
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MARCIE L WOOD  
07/08/2013

**DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH**

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: **204820**

Supporting document/s:	Supp Doc	Letter Date	CDER date
Applicant's letter date:	SD-1	Oct 5 2013	Oct 5 2013
CDER stamp date:	SD-17	Feb 12 2013	Feb 12 2013
	SD-27	May 23 2013	May 23 2013

Product: **Mitigare (colchicine)**

Indication: **Prophylaxis of gout flares in adults**

Applicant: **Hikma Pharmaceuticals, LLC**

Review Division: Division of Pulmonary, Allergy, and  
Rheumatology Products

Reviewer: L. Steven Leshin, D.V.M., Ph.D.

Supervisor/Team Leader: Marcie Wood, Ph.D.

Division Director: Badrul Chowdhury, M.D., Ph.D.

Project Manager: Michelle Jordan-Garner

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# 1 Executive Summary

## 1.1 Introduction

The NDA is for a new formulation of colchicine, a 0.6 mg oral capsule, proposed for the prophylaxis of gout flares. Colchicine has a long history of medicinal use, and was initially approved in 1961 in a fixed-dose combination drug tablet, Colbenemid, containing 0.5 mg colchicine with 500 mg probenecid (NDA 12-383, Merck), followed by a DESI finding of effectiveness for the treatment of chronic gouty arthritis when complicated by frequent, recurrent, acute attacks of gout in 1972. Merck's combination product is no longer marketed. Numerous ANDAs for the Probenecid with Colchicine combination were approved, but only 2 are still marketed (Mirror Pharmaceuticals ANDA 40618; Watson Laboratories ANDA 84279). However, until recently no marketed single-ingredient colchicine product had been approved by FDA. In 2006, the FDA announced a new drug safety initiative to remove unapproved marketed drugs from the market, and bring such products into the FDA approval process to ensure that marketed drugs are demonstrated to be safe and effective for their intended uses. The first single API colchicine oral tablet 0.6 mg was approved in July 2009 (Colcrys, NDA 22-352, Mutual; Takeda since Oct 2012). (b) (4)

For the current NDA submission, West-Ward, the drug manufacturer, collaborated with Hikma to develop and submit a capsule formulation of colchicine.

## 1.2 Brief Discussion of Nonclinical Findings

The applicant submitted published nonclinical literature to support this 505(b)(2) NDA. No new nonclinical studies were conducted or required.

**Pharmacology:** Colchicine binds to the intracellular protein tubulin, preventing its alpha and beta forms from polymerizing into microtubules. This disruption of the microtubular network results in impaired protein assembly in the Golgi apparatus, decreased endocytosis and exocytosis, altered cell shape, depressed cellular motility, and arrest of mitosis. Colchicine also interferes with the formation of the inflammasome, a newly appreciated and identified cellular structure involved in the production of inflammatory-related cytokines. In addition, colchicine also prevents neutrophil migration from the vasculature into tissue by preventing the expression of cell surface adhesion-related molecules E- and L-selectins.

**General toxicology:** Historical information provided by the applicant from published animal and clinical studies indicated similar toxicities with increasing doses. However, the published nonclinical literature contained inadequate long-term toxicology studies. Almost all the studies were conducted prior to GLP regulations and lack much of the information now routinely assessed, such as clinical pathology and histopathology

findings. A direct NOAEL comparison could not be conducted, since nonclinical studies were not conducted with the goal of identifying a NOAEL. Rather, the studies were conducted to identify toxicities or underlying biological mechanisms of colchicine action. In general, the acute toxic signs in animals (rats, dogs, rabbits, cats) with short-term colchicine administration are gastrointestinal tract-related and include emesis, distended intestines, diarrhea (bloody in more severe cases), lack of appetite, and lethargy. With increasing doses these signs become more severe, and there is a loss of body tone, abnormal gait and hindlimb paralysis and wasting atrophy, ascites and eventually death. For comparison, in humans, at doses just above the narrow therapeutic range, colchicine can produce gastrointestinal disorders, profound muscle weakness, respiratory insufficiency, and peripheral neuropathy.

*Genetic Toxicology:* Colchicine was negative for mutagenicity in the bacterial reverse mutation assay. In a chromosomal aberration assay in cultured human white blood cells, colchicine treatment resulted in the formation of micronuclei. However, published studies demonstrated these micronuclei were formed by mitotic nondisjunction without structural DNA changes, and therefore, colchicine is not considered clastogenic.

*Carcinogenicity:* There are no adequate studies of colchicine's carcinogenicity potential. Due to the long history of clinical experience and age of the patient population, carcinogenicity studies were not requested for this application.

*Reproduction and Developmental Toxicology:* The nonclinical literature indicates that colchicine has detrimental effects on reproduction and development due to its inhibition of microtubule formation and cell division. This adversely affects germ cell development by meiosis and subsequent fertility in males and females, as well as interfering with mitosis and subsequent early embryonic development and implantation, and organogenesis. The effects are species and dose dependent, with the timing of exposure also critical for the effects on embryonic development.

Though nonclinical literature indicates that colchicine has detrimental effects on reproduction and development in animals, published clinical epidemiology studies in patients with familial Mediterranean fever found that colchicine therapy during pregnancy was compatible with normal reproduction and developmental in the therapeutic dose range. It is not known if a similar degree of safety is evident in patients taking colchicine for gout, for which the therapeutic dose is usually less than for familial Mediterranean fever. However the patient population with gout is typically beyond the child-bearing age (refer to the clinical review by Dr. Keith Hull).

## **1.3 Recommendations**

### **1.3.1 Approvability**

From the pharmacology toxicology perspective, the application may be approved.

### **1.3.2 Additional Non Clinical Recommendations**

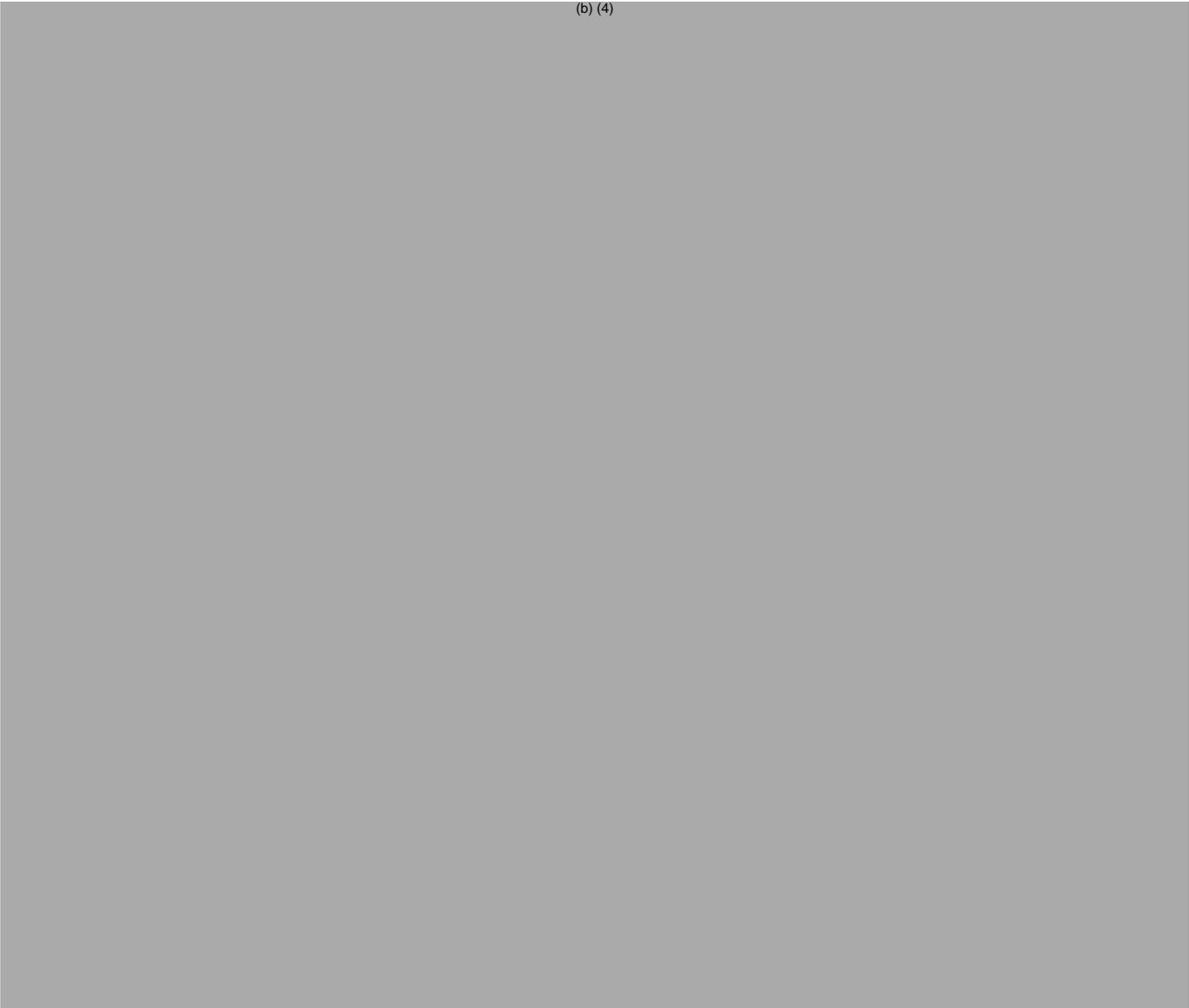
None

### 1.3.3 Labeling

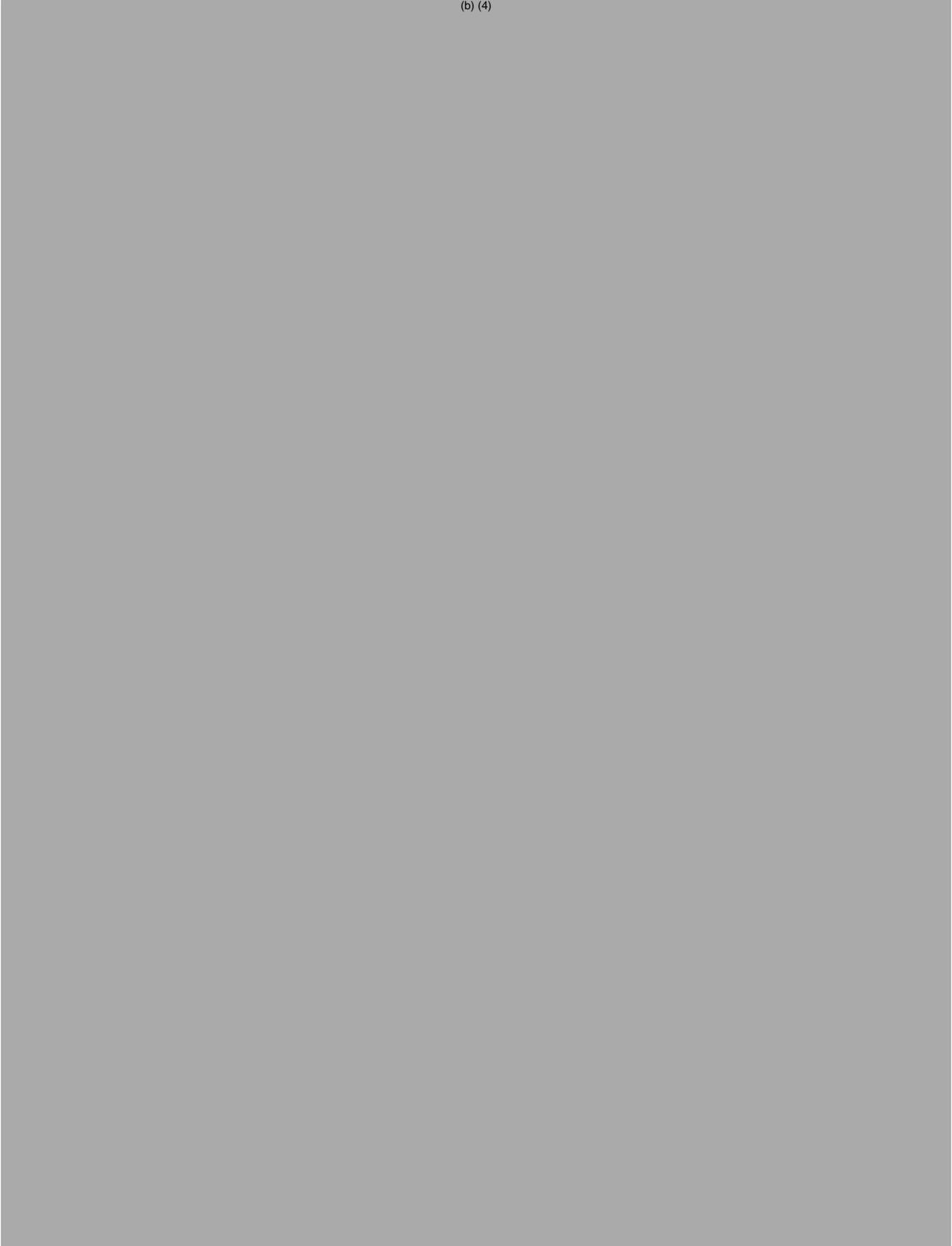
The nonclinical information incorporated into the Mitigare label is similar to the labeling for Colcrys (NDA 22352) labeling since a similar body of published literature was submitted to support the nonclinical aspects of the label for each application. It should be noted that the nonclinical labeling originally submitted to support Colcrys was almost entirely rewritten by the reviewer.

Hikma Proposed Label (from SD-1, Oct 5 2012, and annotated draft labeling of SD-7, Nov 20 2012. Underlined and blue type indicates reviewer additions. Strikeout indicates reviewer deletions.

(b) (4)



(b) (4)



(b) (4)

## 2 Drug Information

### 2.1 Drug

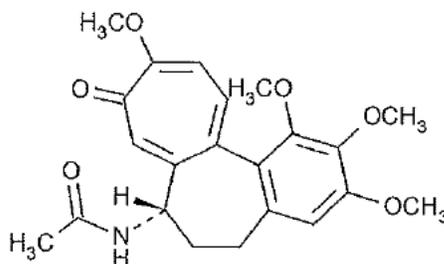
CAS Registry Number : 64-86-8

Generic Name Colchicine

Chemical Name Acetamide, N- (5,6,7,9- tetrahydro- 1,2,3,10- tetramethoxy-9-oxobenzo [alpha]-heptalen-7-yl)

Molecular Formula /  
Molecular Weight  $C_{22}H_{25}NO_6$  /  
399.4

Structure



Pharmacologic Class Not yet established due to uncertainty of its mechanism of action for the treatment of gout (refer to the Pharmacology section for further information)

### 2.2 Relevant INDs, NDAs, BLAs and DMFs

DMF (b) (4) (Colchicine, (b) (4))  
 NDA 12383 (Colbenemid, Colchicine + Probenecid; Merck; approved 1961 and DESI 1972; indicated for the treatment of gout, no longer marketed)  
 ANDA 84279 (Col-probenecid, colchicine and probenecid combination, used as comparator drug in clinical studies, Watson Labs; approved 1976)  
 IND 78601 (0.6 mg colchicine tablets for the treatment of gout; West-Ward Pharmaceutical Co.; submitted Sept 18 2009)

(b) (4)

## 2.3 Drug Formulation

Colchicine capsules, 0.6 mg, consisting of the ingredients listed in the table below. Colchicine capsules are manufactured by West-Ward Pharmaceutical Corp. (Eatontown, NJ).

**Table 1: Composition of Colchicine Capsules, 0.6 mg**

Ingredient	Function	0.6 mg	% Composition
Colchicine, USP	Active	(b) (4)	(b) (4)
Microcrystalline Cellulose, NF (b) (4)	(b) (4)	(b) (4)	(b) (4)
Anhydrous Lactose, NF	(b) (4)	(b) (4)	(b) (4)
Sodium Starch Glycolate, NF (b) (4)	(b) (4)	(b) (4)	(b) (4)
Colloidal Silicon Dioxide, NF (b) (4)	(b) (4)	(b) (4)	(b) (4)
Magnesium Stearate, NF	(b) (4)	(b) (4)	(b) (4)
<b>Total Fill Weight</b>			100.0
Empty Gelatin Capsule Weight			
<b>Total Filled Capsule Weight</b>			

## 2.4 Comments on Novel Excipients

There are no novel excipients. The amounts of excipients are within levels listed within the FDA's Inactive Ingredient Database.

## 2.5 Comments on Impurities/Degradants of Concern

There are six known impurities: (b) (4) (refer to Table 2).

The levels of impurities are within the proposed limits (refer to the CMC review for specific levels). With the exception of the (b) (4), all of the individual specified impurities are limited to the Q3A qualification threshold of 0.15%.

Other known impurities of colchicine include 2 (b) (4), which contain a structural alert (b) (4) for mutagenicity. These are not detected in the clinical product, but standard assay methodologies are not usually sensitive to enable its detection at the recommended

levels of NMT (b) (4). In collaboration with CMC, an IR addressing this issue was sent to the applicant on Feb 4 2013. The applicant provided information in SD-27 (May 23 2013, that included acceptance criterion specifically for (b) (4) of NMT (b) (4)%. The corresponding test methods have also been updated appropriately (refer to the CMC review).

**Table 2: Impurities of Colchicine Drug Substance**

Drug-related Impurity (chemical name or descriptor)	Structure	Origin
(b) (4)		

## 2.6 Proposed Clinical Population and Dosing Regimen

Colchicine is indicated for prophylaxis of gout flares in adults. The dosing regimen is 0.6 mg (one capsule) once or twice daily in adults and adolescents older than 16 years of age. A maximal dose would be 1.2 mg/day. Colchicine capsules are administered orally, with or without food. In patients with impaired hepatic or renal function and in patients who are receiving concomitant treatment with P-gp and CYP3A4 inhibitors, dosage reduction may be necessary.

## 2.7 Regulatory Background

This application is collaboration between Hikma Pharmaceuticals and West-Ward Pharmaceutical Co. West-Ward began marketing 0.6 mg Colchicine Tablets in 1972. West-Ward submitted a PIND meeting package on Sept 19 2007. After receiving the Division's advice on Oct 17, 2007, West-Ward cancelled the meeting scheduled for Oct 18 2007. Subsequently, West-Ward submitted IND 78601 on Sept 18, 2009 to support conduct of clinical comparative bioavailability studies for their 0.6 mg colchicine tablet intended to treat acute gouty arthritis. In these studies, the reference product was a colchicine-probenecid combination tablet. A third comparator arm included probenecid alone to differentiate the colchicine effects of the reference combination product.

Without a preNDA meeting,

(b) (4)



At the July 15, 2011 meeting, West-Ward stated its intention to re-submit a 505(b)(2) application for colchicine based solely on the clinical literature, without any reliance on FDA's finding of safety and effectiveness for Colcrys. A second meeting was held November 30, 2011 to discuss West-Ward's proposed development program for a 0.6 mg capsule formulation (meeting minutes filed Dec 23 2011 under IND 78601).

The applicant subsequently submitted NDA 204820 as a 505(b)(2) submission for 0.6 mg colchicine capsules. Col-Probenecid was used as a comparator drug in the clinical comparative bioavailability studies. There is no currently marketed colchicine capsule.

### **Nonclinical aspects:**

The nonclinical advice provided in the response to PIND 78601 questions (filed Oct 18, 2007) indicated there was insufficient information submitted to determine if nonclinical studies would be necessary, but that both previous human experience and animal studies may be sufficient to assess safety of the product. The sponsor was asked to provide information to support the nonclinical sections of the label or to justify why it

would be unnecessary. At the time of the PIND responses, there were no FDA-approved single entity colchicine products available and the sponsor was directed to distinguish the effects of colchicine from those of the combination product colchicine and probenecid to support the safety and efficacy of their product for the proposed 505(b)(2) application. When IND 78601 was opened, there were no nonclinical concerns with the proposed study, although a PharmTox review was not filed into DARRTS.

After NDA 204820 was filed, an information request was sent to the applicant on Dec 18, 2012 to ask for missing information necessary to support labeling. The requested information was as follows:

1. The following sections of the Module 2 Summaries were indicated as "Not Applicable." Provide information for your product as it relates to drug safety to address these topics. If there is no information available, indicate that. Also provide justification why carcinogenicity studies were not included in your application or not necessary.

Placental Transfer Studies  
Excretion in Milk  
Postnatal Development  
Carcinogenicity

2. Many of the references cited in Module 2 are missing from the publications provided in Module 4. Provide those missing publications.

The applicant submitted the requested information to NDA 204820 on Feb 12, 2013 (SD-17) and adequately addressed the issues.

Also, in collaboration with CMC, the following information request was sent on Feb 4, 2013 to address levels of (b) (4) in the drug substance or conduct toxicologically qualification studies since the (b) (4) have structural moieties that are potentially mutagenic.

1.

(b) (4)

Alternatively, you may conduct in vitro genetic toxicology studies evaluating mutagenicity which, if negative, would support the current proposed specifications.

This was adequately addressed in SD-27 of May 23 2013.

### **3 Studies Submitted**

#### **3.1 Studies Reviewed**

There were no nonclinical studies submitted. A summary with references was provided in Module 2 and most but not all requested references were provided in Module 4. Since the majority of submitted references were clinical in nature, additional supportive nonclinical references, identified by the reviewer, were also incorporated into the review to provide additional clarity concerning pharmacodynamic and toxicological findings.

#### **3.2 Studies Not Reviewed**

The publications were not formally reviewed, but many were examined for support of colchicine's pharmacology and safety. A list of publications, including additional relevant articles identified by the reviewer is listed in the Appendix.

#### **3.3 Previous Reviews Referenced**

None

### **4 Pharmacology**

#### **4.1 Primary Pharmacology**

Colchicine binds to the  $\beta$  subunits of tubulin, thus preventing the polymerization of alpha and beta tubulin forms into microtubules. The disruption of the microtubular network throughout the cell results in impaired protein assembly in the Golgi apparatus, decreased endocytosis and exocytosis, altered cell shape, depressed cellular motility and arrest of mitosis usually in metaphase. Additionally, other studies demonstrated that colchicine interferes with superoxide production in neutrophils (Chia et al 2008) that in turn may alter inflammasome function, a cellular structure involved in the production of inflammatory-related cytokines IL- $1\beta$  and IL-18, and may affect endothelial selectin modulated adhesiveness of neutrophils.

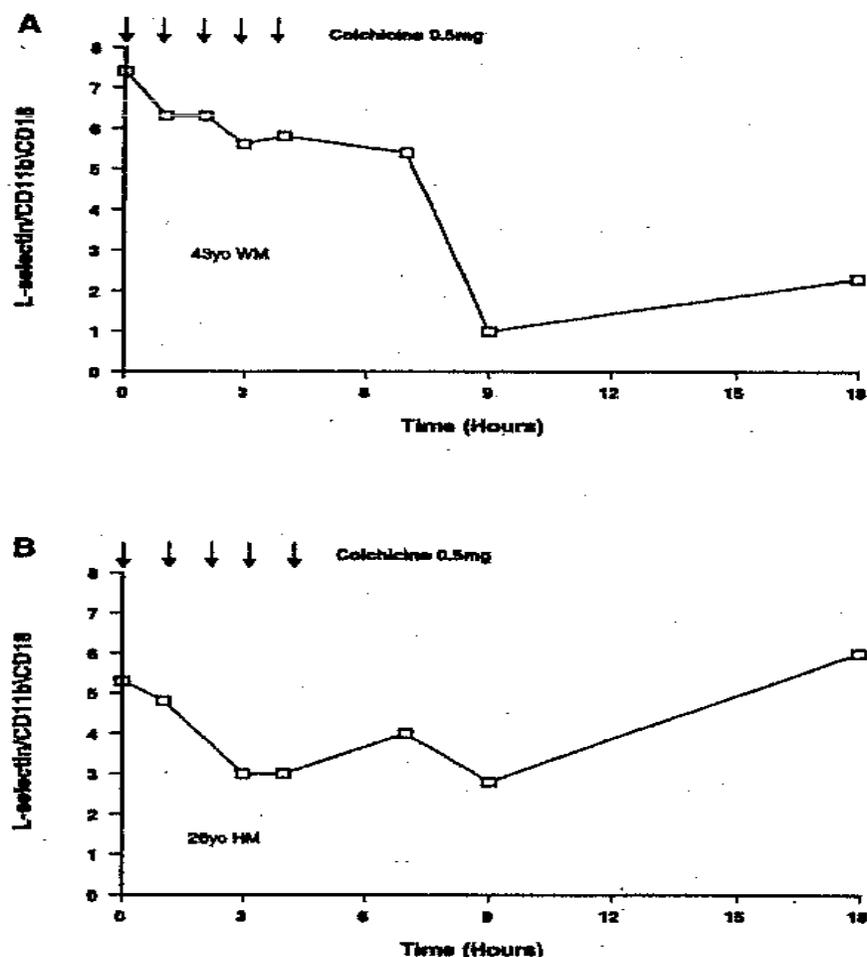
The inflammasome is primarily present in neutrophils and monocytes and is involved in internal cellular surveillance. When activated by the interaction with pyrin protein, this complex activates caspase 1 which then cleaves portions of the inactive cytokines pro-IL- $1\beta$  and pro-IL-18 to produce the active proinflammatory cytokines IL- $1\beta$  and IL-18. (See reviews by Simon and van der Meer, 2007; McDermott and Tschopp, 2007; and Latz et. al., 2013). Colchicine inhibits the migration of mitochondria, the source of caspase 1, to the inflammasome and prevent the inflammasome complex from either forming or functioning, resulting in a reduction of proinflammatory cytokines (Misawa et al 2013). Martinon et al (2006) found that colchicine completely blocked the processing of IL- $1\beta$  in vitro in the differentiated monocytic cell line THP1 in which IL- $1\beta$  was induced by crystals of monosodium urate or calcium pyrophosphate dehydrate. However, the

activation of IL-1 $\beta$  by extracellular ATP was not affected. Similar effects were observed with purified human monocytes. Impaired neutrophil influx was also found in an *in vivo* model of crystal-induced peritonitis in inflammasome-deficient mice or mice deficient in the IL-1 receptor. Their findings indicate that colchicine acts upstream of inflammasome activation.

A study by Cronstein et al. (1995) using freshly collected healthy human leukocytes and umbilical vein endothelial cells, found that extremely low concentrations of colchicine ( $IC_{50} = 3$  nM) prevented neutrophil-endothelial adhesiveness through reduction in endothelial E-selectin expression in response to IL-1 or TNF $\alpha$ . Colchicine could exert its prophylactic effect on cytokine-provoked inflammation by preventing neutrophil migration through the endothelium lining and into the tissue inflammatory sites of activation.

At higher concentrations of colchicine ( $IC_{50} = 300$  nM), the expression of L-selectin was also diminished on the surface of neutrophils. The concentrations of colchicine that inhibit adhesion of neutrophils to endothelial cells (100 nM) are much lower than concentrations which have been reported previously to alter microtubule-dependent functions of inflammatory cells (1  $\mu$ M). Volunteer subjects that were administered oral colchicine (0.5 mg) at hourly interval for a total dose of 2.5 mg (a regimen for the treatment of gouty arthritis) had reductions in neutrophil L-selectin expression, as illustrated for two subjects in the figure below. L-selectin expression returned to baseline 18 h after the last dose in one subject and partially to baseline in the other by the next day.

**Figure 1: Colchicine treatment diminishes L-selectin expression relative to CD11b/CD18 on the surface of peripheral blood neutrophils (Cronstein et al., 1995).**



### Activity of Colchicine Isomers and Metabolites

The isomer ( $\alpha$ S, 7S) of the 4 potential colchicine forms is only one that binds tubulin. High affinity binding requires that colchicinoids exhibit the proper stereochemical arrangement of the A and C rings. The affinity of colchicine for the colchicine site on tubulin is directly related to the effectiveness in inhibition of microtubule polymerization. The dissociation of colchicine from tubulin is slow and therefore its action is considered essentially irreversible. Bound colchicine alters the lateral contacts between tubulin subunits preventing the straightening of the curved tubulin shape. At low concentrations of colchicine, the number of missing lateral contacts is small and the microtubule mass is preserved, although some of the normal cellular functions might be disrupted. At high colchicine concentrations, the proportion of missing lateral contact increases, the ends destabilize, and the microtubule mass disassembles and disappears from the cell.

The anti-inflammatory activity of two primary metabolites of colchicine, 2-

demethylcolchicine (2-DMC) and 3-demethylcolchicine (3-DMC), were tested *in vivo* using the rat carrageenin-induced footpad edema model (100 µg injected of each compound/foot; Sugio et al, 1987). 2-DMC did not inhibit edema but 3-DMC was as effective as colchicine. Specifically, at 3 and 5 hours after injection, 3-DMC inhibited edema by 39 and 47% and colchicine inhibited edema by 44% and 53%. Also Dvorak et al (2007) demonstrated that colchicine and 3-DMC had similar potency profiles on human hepatocytes in culture. With increased dose both compounds increased cellular damage (assessed by an increase in lactate dehydrogenous leakage) and reduced albumin secretion by approximately the same percentage compared to control vehicle treated cultures.

## 4.2 Secondary Pharmacology

Microtubules are cytoskeletal polymers of tubulin involved in many cellular functions. They not only serve a physical role in providing the cytoskeletal structure but they also are critical for intracellular movement of organelles, vesicles, surface transporters and receptors, and chromosomes. Some of the specific toxicological sequelae that arise from colchicine's interference with these cellular processes are described in the following section, Section 4.3, Safety Pharmacology.

## 4.3 Safety Pharmacology

Findings from published nonclinical studies closely match those known from the published extensive clinical experience with colchicine. Adverse effects are primarily detected in gastrointestinal, muscle, and some hematopoietic cells. Dvorak et al. (2007) suggested that since the biological activity of colchicine is tightly bound to its inhibitory effects on tubulin polymerization, its side effects are most prominent in proliferating cells.

### Neurological and Muscular Effects

Colchicine does not normally cross the blood-brain-barrier to any meaningful extent since the brain-to-blood ratio of colchicine after systemic administration is very low due to efflux by the P-gp transporter. However, high concentrations of colchicine administered peripherally can result in seizure followed by death in rodents although it is unclear if this is a direct effect or a secondary effect from blocking axonal transport. Direct injection of colchicine into the hippocampus of rats and monkeys caused a non-specific inflammatory response resulting in nerve destruction that is both dose- and species-dependent (Dasheiff and Ramirez, 1985).

In animals, colchicine induced muscle weakness, changes in gait, and body positioning have been associated with alterations in skeletal muscle and peripheral neuropathy, with more severe effects at higher dose (Markand et al., 1971; Seiden 1973; Chang et al 2002. In humans, Kuncl et al (1987) reported muscle weakness and ascending delayed paralysis in patients with gout as well as altered renal function.

**Table 3: Neurological and Muscular Effects**

Species / Dose of Colchicine	Findings
<p><b>Markand et al. 1971</b></p> <p>Rats 0.4 mg/day (~1.4 to 1.6 mg/kg), i.p., (Note: this dose resulted in death in approximately one-third of the animals)</p>	<ul style="list-style-type: none"> <li>• Induction of paralysis in rats was secondary to myopathic rather than neuropathic alterations.</li> <li>• In the surviving animals sacrificed 3 to 4 days after colchicine injection, extensive damage observed by light microscopy consisted of disruption and degeneration of myofibrils, large necrotic zones which lacked myofibrils but contained numerous membranous bodies, and amorphous sarcoplasmic debris.</li> <li>• Electron microscopy revealed changes as early as 24 hours after injection of colchicine. The earliest change occurred in the sub-sarcolemmal area, followed by focal alterations in the intermyofibrillar zones. The most conspicuous change on the second and third day was the accumulation of large sarcoplasmic membranous bodies of varying size and complexity. Some contained small vesicles, osmiophilic granules, and mitochondria in the center. Others had several concentric layers of membranes and resembled myelin (some had mitochondria enclosed in the membranes). Nuclear changes were also observed.</li> <li>• No ultrastructural changes observed in the muscles of control animals or in sections of sciatic nerve or anterior horn cells of the spinal cord from treated animals.</li> </ul> <p>Hypothesis: Colchicine interferes with lysosomal degeneration.</p>
<p><b>Seiden (1973)</b></p> <p>Sprague-Dawley rats, adult males 0.4 or 0.8 mg/kg/day, i.p., daily for 2 to 22 days</p>	<p>No gross changes were observed in the muscle of treated animals at lower doses over a longer dosing period</p> <ul style="list-style-type: none"> <li>• 0.4 mg/kg/day: mild toxic effects or none at all.</li> <li>• 0.8 mg/kg/day: severe toxic effects including weight losses up to 27% of body weight within 4 days, diarrhea, weakness, and paralysis.</li> </ul> <p>Ultrastructure: no evidence of myofibrillar degeneration, but changes in myofilament orientation and the appearance of many unusual membranous structures. Disoriented filaments appeared in a sub-sarcolemmal position, primarily in perinuclear zones that were devoid of normally oriented myofilaments, but rich in sarcoplasm, mitochondria, and other organelles and spheromembranous bodies.</p>
<p><b>Chang et al. 2002</b></p> <p>Sprague-Dawley rats, females 0.2 mg/kg, i.p., daily for 5 days/week, for either 7 months or 10 months; control rats were</p>	<ul style="list-style-type: none"> <li>• Gait abnormalities observed during monthly walking-track analysis.</li> <li>• No differences in rectus femoris muscle biopsies</li> </ul>

<p>untreated</p> <p>Rectus femoris muscle biopsies and nerve biopsies were obtained at 7 and 10 months</p>	<p>between treated or control animals from analysis by phase or electron microscopy.</p> <ul style="list-style-type: none"> <li>• No vascular autophagic changes in the colchicine-treated group.</li> <li>• Nerve biopsies showed no differences in the sciatic and posterior tibial nerve fibers.</li> <li>• Statistical significant difference (<math>P &lt; 0.001</math>) in the axon / myelin ratio of the sciatic nerve in colchicine-treated animals versus controls.</li> </ul> <p>Conclusion: Chronic colchicine administration, in doses that produce weakness and some weight loss but not diarrhea, altered neuromuscular function (changes in gait) without producing measurable changes to muscle and nerve fibers.</p>
<b>Kuncl et al., 2003</b>	
<p>Rats</p> <p>0.4 mg/kg, i.p., daily for 4 weeks</p> <p>The effects of colchicine on acetylcholine receptor (AChR) trafficking were studied in cultured myotubes.</p>	<p>Rat model for human myopathy:</p> <p>Evidence supporting a functional role of microtubules in the acetylcholine receptor trafficking and lysosomal degradation in normal adult skeletal muscle, and this is disrupted in colchicine myopathy.</p> <p>Colchicine injection resulted in chronic proximal weakness along with skeletal muscle changes that are consistent with subacute myopathy seen in humans (e.g., vacuolar changes in non-necrotic myofibers). In immunolabeling ultrastructural studies, colchicine inhibited exocytosis and the overall degradation of membrane receptors which was correlated with disruption of the membrane subsurface tubulin network.</p>

### Gastrointestinal Effects

Gastrointestinal side effects reported clinically include abdominal cramping, abdominal pain, diarrhea, lactose intolerance, nausea, vomiting, and elevated serum enzymes AST and ALT. Gastrointestinal effects are among the most prevalent adverse events observed with therapeutic doses in humans. Similar effects are observed in animal studies, with vomiting and diarrhea to bloody diarrhea usually the first signs noted. These effects are due to a combination of factors, including enhanced intestinal permeability, inhibition of water transport due to decreased activity of intestinal Na<sup>+</sup>-K<sup>+</sup>-ATPase, and disruption of cytoskeletal integrity in the more rapidly dividing cells of the gut and peripheral activation of central processes mediating emesis.

**Table 4: Gastrointestinal Effects of Colchicine**

Species / Dose of Colchicine	Findings
<b>Ferguson, 1952</b>	
<p>Wistar rats, male and female 0.5, 1, 2, 4 mg/kg, iv 4 mg/kg, ip</p> <p>Cats, male and females, Acute tox IV doses were 0.12, 0.25, 0.5 and 1.0 mg/kg in conscious or anesthetized animals, (urethane or pentobarbital dose of 0.1, 0.25, 0.5, 1 mg/kg, iv or ia, or inhalation ether) Chronic tox, IP doses 0.25 mg/kg/day, at 5-days/week, dose doubled each week for 4 weeks)</p>	<ul style="list-style-type: none"> <li>• Altered gastrointestinal responses to colchicine were observed, depending on whether animals were conscious or anesthetized, suggesting that emetic actions are at least partially centrally mediated.</li> <li>• Consistent emetic effects were observed in unanesthetized animals, but only a fourth of the anesthetized cats vomited after a lethal dose anesthesia.</li> <li>• Anesthesia eliminated the diarrhea that normally followed administration of colchicine</li> <li>• Doses of colchicine from 0.1 to 1.0 mg/kg had no effect on intestinal motility or tone in cats that manifested as either spontaneous motility or hypermotility caused by neostigmine</li> <li>• Signs of increased tone and increased rate and amplitude of contractions were observed in Thiry-Vell loops of the small intestines in conscious cats. These effects appeared a few hours after injection of colchicine, persisted for hours, and could not be completely abolished by atropine.</li> <li>• Bowel responses to acetylcholine, epinephrine, and histamine were unaffected by colchicine <i>in vivo</i>, and exposing isolated strips of bowel to colchicine in physiological ranges produced no effect on normal motility.</li> </ul>
<b>Dinsdale, 1975</b>	
<p>Hooded Lister rats, male 0.1 to 4.0 mg/kg, s.c. Cytosine arabinoside hydrochloride (125 to 500 mg/kg, s.c.) Cycloheximide (1.0 mg/kg, s.c.) 30 min prior to colchicine</p> <p>Duodenum tissue obtained 6 hrs postdose</p>	<p>Colchicine induced dose-dependent necrosis of rapidly proliferating cells of the duodenal crypts and the mature cells of the gastrointestinal villi.</p> <ul style="list-style-type: none"> <li>• At less than 0.2 mg/kg: indistinguishable from controls at the light microscope level.</li> <li>• 4 mg/kg: lethargy during postdose hour 5-6, gas filled stomach</li> <li>• At 2 mg/kg (the ~LD<sub>50</sub>: based on Ferguson and Theodore, 1952), effects were restricted to the crypts of the mucosa and essentially absent from the mature cells of the villi and the adjacent pancreatic tissue by light and electron microscopy.</li> </ul>

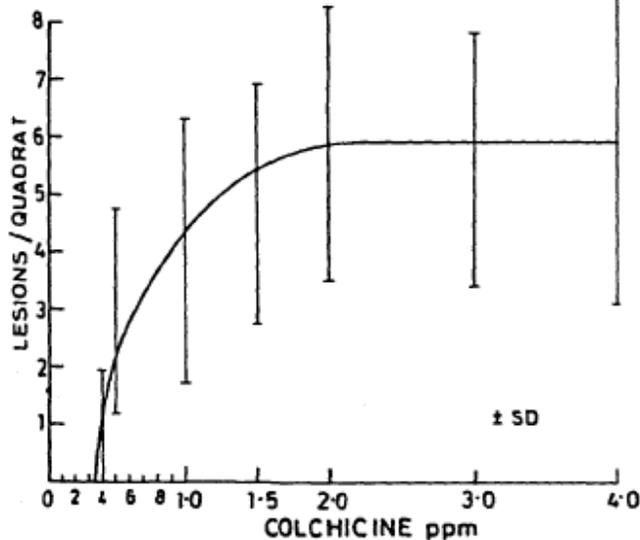


Fig. 2—The incidence of lesions in the duodenal crypts of rats sacrificed six hours after injection with different doses of colchicine

- EM: Colchicine-induced inclusion bodies were observed. They were variable in size (0.5 to 8.0 μm) contained mitochondria and had a limiting membrane, but no nuclear remnants or golgi apparatus.
- Cycloheximide (protein synthesis inhibitor) greatly reduced the severity of damage to the duodenal crypts but did not prevent the arrest of mitosis by colchicine. The reduction in damage due to cycloheximide was attributed to interference with the cellular response to injury that requires synthesis of proteins.

**Rachmilewitz *et al.*, 1978,  
Rachmilewitz and Karmeli, 1980**

Male rats  
5 mg/kg, i.p.

Jejunal segments obtained 1, 2, or 4 hours after injection for assays

Histological sections from jejunum obtained 4 hours after administration

- Colchicine inhibited intestinal water transport and intestinal fluid secretion mediated through decreased intestinal  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  activity, increased adenylate cyclase and cAMP activities, and increased prostaglandin E2.
- Net transport of fluid in the jejunal segments was significantly ( $P < 0.01$ ) reduced in rats that had received colchicine ( $3.0 \pm 0.9$  g fluid/hour/g) *versus* the control rats ( $8.6 \pm 0.7$  g fluid/hour/g). Decreases in net fluid transport were paralleled by the decreases observed in  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  activity. Along with the decrease in net fluid transport, reduced amounts of sodium and potassium were transported.
- Histologically, the jejunum from rats treated with colchicine was relatively normal. The only unusual finding was the appearance of crypt cells undergoing mitotic arrest in metaphase.
- Four hours after administration of colchicine,  $\text{Na}^+ - \text{K}^+ -$

	ATPase activity was significantly ( $P < 0.01$ ) decreased - ( $18.2 \pm 4.9 \mu\text{mol/mg protein/hour}$ ) as compared with control rats ( $40.6 \pm 3.4 \mu\text{mol/mg protein/hour}$ ).
<b>Duncan and Heddle (1984)</b>	
C57BL/6J mouse colchicine IV in saline doses: 0, 10, 20, 40, and 80 $\mu\text{g}$ to 23-23 g mice Tissue obtained 6 hr post injection	Apoptosis occurred in duodenal crypt enterocytes.  Apoptosis occurred with a gradient more frequently at the crypt bottom than the crypt top and in association with the location of similar percentages of metaphase cells.
<b>Fradkin et al. (1995)</b>	
Wistar rats, Colchicine dissolved in their drinking water at 30 mg/L Studies of 8 and 23 days duration  Rats consumed 16.4 mL in the first 8 day, and 19.2 mL in the 23-day studies.  Calculated dose per animal = $0.5 \pm 0.15$ mg/day. Day 23 serum colchicine concentrations $3.8 \pm 2.7$ ng/mL (range 1.0-6.7 ng/mL)	Colchicine increased tight junction permeability in the rat as evidenced by the sustained increase in lactulose/ mannitol excretion ratio. <ul style="list-style-type: none"> <li>• The lactulose/ mannitol excretion ratio was measured periodically</li> <li>• This type of double-probe method for measuring intestinal permeability controls for such variables as transit time, renal function and completeness of urine collection.</li> <li>• Mean serum colchicine concentration in the rats completing 23 days of administration was 3.82 ng/mL (comparable to those recorded in FMF patients receiving 1-2 mg/day colchicine therapy).</li> <li>• Except for occasional loose stools, diarrhea was not observed. Two of 5 animals were removed from the 23-day study in the last week, suffering from severe anorexia and bloody nasal discharge.</li> <li>• Urinary lactulose excretion increased significantly at 8 hr post-dose compared to pretest values and was highest after 48 hours, remaining stable throughout the study period. Mannitol urinary excretion was not changed.</li> </ul>
<b>Iacobuzio-Donahue et al., 2001</b>	
Humans, clinically toxic doses, distinct morphologic changes are observed in gastrointestinal mucosal biopsies of the duodenum and gastric antrum	Changes include metaphase mitoses, epithelial pseudo-stratification, and loss of polarity as well as abundant crypt apoptotic bodies (See table 2 below).  Changes were not observed in 5 patients without clinical colchicine toxicity.

**TABLE 2.** Histopathologic and endoscopic features of gastrointestinal biopsy specimens from patients on oral colchicine therapy

Case no.	Biopsy site	Metaphase mitoses	Epithelial pseudostratification	Loss of polarity	Apoptosis	Villous atrophy	Endoscopic findings
<b>Patients with clinical evidence of colchicine toxicity</b>							
1	Duodenum bulb	++	++	++	++	-	Duodenal polyp and ulceration
	Antrum	++	++	++	++	-	Diffuse gastritis
2	Body	+	+	+/-	+	-	Normal
	Duodenum	++	-	-	++	-	Normal
3	Antrum	++	+	+	++	-	Normal
	Body	+/-	-	-	-	-	Normal
4	Antrum	++	++	+	+	-	Diffuse gastritis and erosions
	Fundus	-	-	-	-	-	Normal
5	Colon	++	++	+/-	-	-	Normal
	Duodenum	++	+	+	-	+	NA
<b>Patients without clinical evidence of colchicine toxicity</b>							
5	Colon	-	-	-	-	-	Polyp
6	Antrum	-	-	-	-	-	Mild gastritis
	Esophagus	-	-	-	-	-	Normal
	GE	-	-	-	-	-	Normal
	Junction	-	-	-	-	-	Polyp
7	Colon	-	-	-	-	-	Polyp
	Duodenum bulb	-	-	-	-	-	NA
	Antrum	-	-	-	-	-	NA
8	Esophagus	-	-	-	-	-	NA
	Antrum	-	-	-	-	-	NA
9	Colon	-	-	-	-	-	NA
	Colon	-	-	-	-	-	Polyp

NA, not available.

**González et al., 2005**C3H/S mice, adult male,  
2 mg/kg, i.p.,Sacrifice at 4 hours postdose Duodenum  
histopathology and TUNEL assay (nick  
end-labeled for DNA fragmentation)

Colchicine induced apoptosis in intestinal crypt enterocytes

- Cell was considered to be apoptotic if cell shrinkage, intense eosinophilic cytoplasm, and a nucleus with condensed chromatin were observed.
- The average apoptotic indices in the whole crypt and each individual region were higher ( $P < 0.0001$ ) in colchicine-treated mice than in saline-treated control mice.
- For the whole crypt the apoptotic index in saline treated animals was  $2.12 \pm 0.58$  and for colchicine-treated mice, it was  $65.73 \pm 9.09$ . The highest apoptotic index was seen in tiers 5-12 (transient cells) for saline ( $3.14 \pm 0.94$ ) and colchicine-treated ( $107.59 \pm 10.72$ ) animals. The lowest apoptotic index (the number of apoptotic cells per 1000 nuclei) seen was in tiers 13-20 (highly differentiated cells). In this zone, the apoptotic index in the saline-treated animals was  $1.01 \pm 0.28$  and in the colchicine-injected mice, it was  $34.77 \pm 10.81$ .

**Hematopoietic Effects**

Myelosuppression is a known clinical dose-related adverse event associated with colchicine due its effects on disruption of mitosis in rapidly dividing cells such as is found in the hematopoietic system. A dose dependent leukopenia due to reductions in polymorphonuclear cells, with a more mild lymphopenia in rabbits and dogs was demonstrated over 100 years ago by Dixon and Malden (1908).

Studies in human granulocytes (e.g., neutrophils, eosinophils, basophils) lacking in

phospho-glycoprotein (P-gp) contain concentrations of colchicine that are higher than cells that express P-gp on their surface (Chappey et al (1995). As noted by the Applicant, these observations are consistent with the hypothesis that the anti-inflammatory effect of colchicine is mediated by neutrophils (Ben-Chetrit and Levy, 1998). These cells also might be more susceptible to the toxic effects of colchicine and could account for adverse events observed in the hematopoietic cells in humans.

## 5 Pharmacokinetics/ADME/Toxicokinetics

Almost all nonclinical toxicology studies lacked the toxicokinetic information to correlate blood concentrations with specific toxicities, and more recent toxicokinetic studies lacked toxicity information. Some metabolites of colchicine have biological activity, but whether they are present in blood has not been determined in animal studies. Colchicine was an essential instrument for understanding the role of membrane transporter compounds. Colchicine was used to initially identify and isolate its major transporter, P-gp, which is involved in colchicine absorption, distribution and excretion, and especially in maintaining low brain concentration of colchicine, the secretion of colchicine into bile, and colchicine secretion into kidney tubules.

### Absorption and Distribution

Colchicine distributes into multiple tissues but primarily into bile, liver, and kidney summarized in the tables that follow. Moderate levels were detected in the lungs, heart, intestine, and stomach.

In 10-day old rats, the tissue concentrations of colchicine were much higher than were observed in 35-day old animals for a given dose (Hunter and Klassen, 1975b). The authors suggested that, in the young rats, colchicine elimination is reduced due to an immature bile duct tract. Reduced elimination, in turn, results in higher systemic and tissue levels and a lower LD<sub>50</sub> (0.24 mg/kg, i.p.) than seen in 35-day old animals (LD<sub>50</sub> 2.0 mg/kg, i.p.).

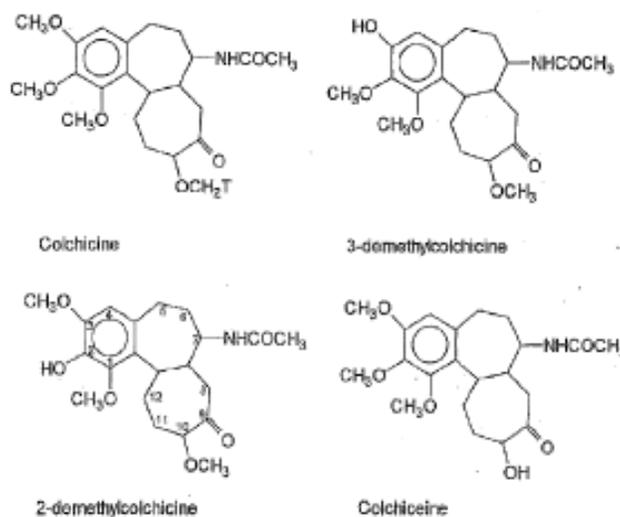
Despite a relatively large volume of distribution, only small amounts of colchicine have been shown to penetrate the brain of rats, hamsters, and rabbits (Hunter and Klassen, 1975a; Bennett et al., 1981). Limited brain uptake is thought to be due to P-gp mediated efflux at the blood-brain barrier. In rats, P-gp efflux normally reduces the penetration of colchicine into the brain and co-administration of a P-gp inhibitor (e.g., verapamil or PSC-833) can result in increased brain levels (Desrayaud et al., 1997).

### **Metabolism**

Colchicine is metabolized in liver microsomes and the extent varies across species. In hamster, ~40% of colchicine is converted into metabolites, but in rat and mouse liver only about 11 to 12% is metabolized (Schönharting *et al.*, 1974). The specific CYP450 enzymes in nonclinical species have not been defined. In human liver microsomes, the primary metabolizing CYP enzyme is CYP3A4 (Tateishi et al., 1997). The primary

metabolites formed in liver microsomes of all species studied are 2-DMC and 3-DMC. In rats and hamsters, colchicine is metabolized to O-demethyl derivatives which are then glucuronidated. In rat hepatocytes *in vitro*, colchicine inhibits the expression of multiple CYP enzymes and associated regulatory co-factors.

**Figure 2: Metabolites of Colchicine (from Tateishi et al 1996)**



Plasma concentrations of metabolites have not been measured in animals. In rats, *in vivo*, 2-DMC does not inhibit inflammation but 3-DMC is as active as colchicine (Sugio et al., 1987). It is not known if these metabolites are substrates of P-gp. Glucuronides are present in the rat (2-DMC-glucuronide) and hamster (3-DMC-glucuronide).

Injury to the liver impairs the clearance of colchicine in rats *in vivo* (Leighton et al. 1990). In addition, colchicine inhibits many aspects of liver function. In rats, the administration of colchicine at doses that are approximately half the LD<sub>50</sub>, colchicine reduces the hematocrit, glutathione (GSH), total CYP450 content, and demethylase activity, and increases liver mass, serum AST, ALT, and lipid peroxidation activity. Co-administration of CYP inhibitors to the rat resulted in ~3-fold increase in colchicine liver concentrations, while co-administration of CYP inducers to hamster resulted in ~3- to 4-fold increase in colchicine metabolism.

In hamsters, CYP inducers (20-methylcholanthrene and phenobarbital) produced several fold increases in the metabolism of colchicine *in vitro*. Colchicine also induced an increase in its metabolism but to a much lower extent (by approximately 0.5- to 0.3-times compared to the above CYP inducers (Schönharting et al., 1974). In comparison to other species, mice and rats, colchicine is more extensively metabolized in hamsters and has a different metabolic profile (Schönharting et al., 1974).

## Excretion

The clearance of colchicine is mediated by liver metabolism and secretion into the bile and urine. Colchicine is excreted primarily into the feces (70%) and, to a lesser extent,

into the urine (14%) in rats by 96 hours after a single administration, with most of this excreted within the first 24 hours (56% in feces, 11% in urine; Hunter and Klaassen, 1975). Demethyl metabolites and other unidentified polar metabolites are also excreted in the bile.

Blood colchicine is not only filtered into the kidney, but is actively excreted. Excretion of colchicine into bile or urine appears to be primarily mediated through P-gp transport. Speeg et al. (1992) demonstrated that cyclosporin, a P-gp inhibitor, inhibited colchicine excretion into urine. At high doses, in the rat, colchicine administration alters the normal function of transporters and CYP enzymes.

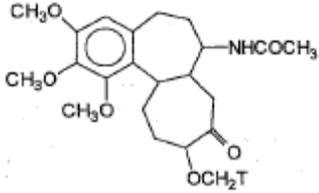
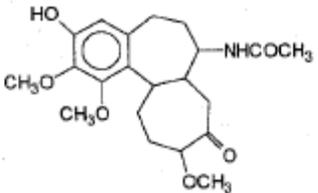
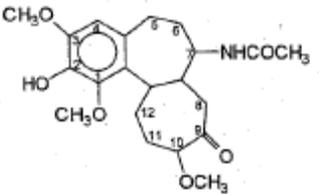
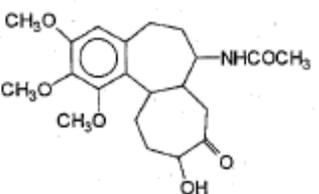
Non-absorbable fat substitutes sucrose polyester (SPE) and tricarballylate triester (TCTE) reduced biliary reabsorption of colchicine in rat without altering its biliary excretion (Benmoussa et al., 1995)

### Pharmacokinetic drug interactions

The sponsor reviewed the literature and together with their clinical studies of CYP enzyme substrate interaction indicated that drugs that are substrates of P-gp transporters and CYP3A4 may affect colchicine pharmacokinetics and therefore the safe use of colchicine. The applicant noted that synergistic pharmacologic effects could contribute to the reported interactions involving cases of myotoxicity. Drug interaction studies were conducted by the applicant and this information is can be found in the Clinical Pharmacology Review by Dr. Sheetal Agarwal.

**Table 5: Colchicine ADME Studies**

Methods	Findings
<p><b>Modriansky and Dvorak (2005)</b> Review discusses the effect of four well known natural antimetabolites: colchicine, taxol (paclitaxel), vincristine, and vinblastine, and a synthetic microtubule disruptor nocodazole on transcriptional activity of glucocorticoid and aryl hydrocarbon receptors, regulators of CYP enzyme expression.</p>	<p>Microtubules network perturbation alters transcriptional activities of AhR and GR receptor that are known to be involved in CYPs regulation.</p> <ul style="list-style-type: none"> <li>• Microtubules disarray restricts signaling by two nuclear receptors, the glucocorticoid and aryl hydrocarbon receptors, regardless of cell cycle phase.</li> <li>• Two colchicine metabolites arising due to CYP3A4 activity have been identified: 2-demethylcolchicine and 3-demethylcolchicine. 2-Demethylcolchicine is much less potent whereas 3-demethylcolchicine is comparable to its parental drug in terms of microtubule disruption.</li> <li>• In primary cultures of human hepatocytes treated with colchicine resulted in glucocorticoid receptor-mediated down-regulation of CYP2B6, CYP2C8, CYP2C9, and CYP3A4. Colchicine restricted nuclear import of GR in human hepatocytes and in human embryonal kidney cells (HEK293) transiently transfected with GR-GFP chimera1.</li> <li>• Colchicine derivative colchicine (10-O-demethylcolchicine), which lacks tubulin-binding capability, had no effect.</li> </ul>

	<ul style="list-style-type: none"> <li>• Microtubules interfering agents decreased both basal and rifampicin- and phenobarbital-inducible expression of these CYPs,</li> </ul>
<p><b>Tateishi, 1997</b></p> <p>Biotransformation of <math>^3\text{H}</math>-colchicine in a panel of microsomal preparations obtained from sixteen human liver samples</p>	<p>Colchicine is metabolized by human liver microsomes to the 2- and 3-desmethyl colchicine (2DMC, 3DMC) derivatives, by CYP3A4, but not CYP2A6, CYP2C19, CYP2C9, CYP2D6, and CYP2E1.</p> <p>Both parent drug and metabolites are found in significant amounts in bile, indicating enterohepatic recirculation.</p>
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>Colchicine</p> </div> <div style="text-align: center;">  <p>3-demethylcolchicine</p> </div> </div> <div style="display: flex; justify-content: space-around; align-items: center; margin-top: 20px;"> <div style="text-align: center;">  <p>2-demethylcolchicine</p> </div> <div style="text-align: center;">  <p>Colchicine</p> </div> </div>	
<p><b>Desrayaud et al., 1997</b></p> <p>Male Hanover Wistar rats,</p> <p>Colchicine administered iv (femoral vein) alone or together with the P-gp inhibitor, PSC-833</p> <p>Simultaneous microdialysis of blood (jugular vein) and brain (front cortex) of conscious, freely moving rats. Vehicle or SDZ PSC-833 bolus dose (2.3 mg/kg, i.v.), followed by a 7-hour infusion (16.7 <math>\mu\text{g}/\text{min}/\text{kg}</math>). One hour after starting the SDZPSC-833 treatment, colchicine was administered initially as a bolus dose (1 mg/kg, i.v.), followed by a 2-hour infusion (12.5 <math>\mu\text{g}/\text{min}/\text{kg}</math>).</p> <p>(SDZ PSC 833 is a cyclosporin D analog that reverses multi-drug resistance)</p> <p>Blood and brain dialysate samples collected every 20 minutes during the infusion and for 4 hours after initiating the infusion.</p>	<p>Control group: Brain colchicine concentrations were below the limit of detection.</p> <p>PSC-833 pretreatment: Brain colchicine concentrations were significantly greater for the first hour after administration. Thereafter, no significant differences were observed between the two groups.</p> <p>Relative to the control group, intravenous SDZ PSC-833 co-administration increased the brain exposure of colchicine (<math>\text{AUC}_{0-6}</math>, brain) at least 10-fold and blood exposure of colchicine (<math>\text{AUC}_{0-6}</math>, blood) increased <math>\sim 2.5</math>-fold.</p> <p>Effect of SDZ PSC 833 on blood disposition parameters of free colchicine</p>

Unbound colchicine concentrations were measured in brain and blood dialysate samples <i>via</i> RIA.		Control group (n = 4)	SDZ PSC 833 treated group (n = 3)	
	$C_{ss}$	ng/ml	149.6 ± 9.9	333.5 ± 81.7*
	AUC <sub>0-6h</sub>	µg/ml*h	0.51 ± 0.02	1.28 ± 0.34*
	CL	ml/min/kg	80.4 ± 5.8	42.6 ± 10.0*
	$V_{d\beta}$	l/kg	21.4 ± 4.2	17.6 ± 7.7
	$t_{1/2\alpha 1}$	h	0.22 ± 0.03	0.34 ± 0.07
	$t_{1/2\beta}$	h	3.04 ± 0.47	4.52 ± 1.08
Data are means ± SEM. * p < 0.05 vs control in Student's t test.				
Effect of SDZ PSC 833 on the Blood and Brain Disposition Parameters of Free Colchicine.				
		Control group (n = 4)	SDZ PSC 833 treated group (n = 3)	
AUC <sub>blood 0-6h</sub>	µg/ml*h	0.51 ± 0.02	1.28 ± 0.24*	
AUC <sub>brain 0-6h</sub>	µg/ml*h	≤ 0.02 ± 0.01	0.20 ± 0.05*	
$K_{brain/blood}$		≤ 0.04 ± 0.01	0.15 ± 0.06*	
Data are means ± SEM. * p < 0.05 vs control in Student's t test.				
<b>Bennett et al (1981)</b>				
Mice and rats uptake of [ring A-4- <sup>3</sup> H] colchicine and [ring C-methoxy- <sup>3</sup> H]colchicine in mice up to 24 hr postdose	<p>Less radioactivity in brain after administration of ring-labeled colchicine than after administration of the methoxy-labeled colchicine.</p> <p>Amount of colchicine entering mouse brain after subcutaneous injection is comparable to the minimum behaviorally effective dose when administered to the caudate nucleus.</p> <ul style="list-style-type: none"> <li>• 3 hr after administration of ring-labeled colchicine, 5% of the label was in liver and about 0.01% of the label was present in brain.</li> <li>• 40% of the brain radioactivity was bound to tubulin as determined by vinblastine precipitation.</li> <li>• After 3 hr, an average of 8% of the radioactivity from methoxy-labeled colchicine was found in the liver and 0.16% in brain.</li> <li>• &lt;5% of the activity in brain was precipitated by vinblastine, and the colchicine equivalent was comparable to that found after administration of the ring-labeled colchicine.</li> <li>• The general pattern in rats was similar to mice: less radioactivity was found in brain after administration of the ring labeled alkaloid than after administration of methoxy labeled colchicine.</li> <li>• 4% to 50% of ring~labeled colchicine was precipitated by vinblastine and a smaller percentage of the methoxy-labeled drug was precipitated by vinblastine than the ring A-labeled colchicine.</li> </ul>			

TABLE I  
RADIOACTIVITY IN BLOOD, LIVER AND BRAIN OF MICE AFTER SC ADMINISTRATION OF  
[Ring A-4-<sup>3</sup>H] COLCHICINE

	nmoles Adm	% in Blood*	% in Liver	% in Brain	% of Brain Supernatant		Colchicine Equivalent in Brain† (pmoles)
					TCA Insoluble	Vinblastine Precipitable	
1 Hr Incorporation Time (2 experiments)							
Average	3.8	0.94%	6%	0.008%	3%	43%	0.14
Range	(3.6-4.0)	(0.68-1.2)	(5-7)	(0.007-0.009)	(2-4)	(42-44)	(0.12-0.16)
3 Hr Incorporation Time (7 experiments)							
Average	4.1	0.26%	5%	0.009%	3%	40%	0.14
Range	(3.5-5.4)	(0.12-0.53)	(2-9)	(0.007-0.011)	(1-4)	(26-54)	(0.10-0.19)
24 Hr Incorporation Time (1 experiment)							
	4.0	0.22%	0.3%	0.012%	1%	10%	0.05

\*The blood volume was assumed to be 78 ml/kg mouse [24]; body weights were  $34 \pm 3$  g, and livers averaged 2 g.

†The colchicine equivalent was calculated from percentage of brain supernatant radioactivity precipitated by  $2.5 \times 10^{-3}$  M vinblastine multiplied by total radioactivity in brain. The result was multiplied by 4 and divided by the actual nmoles injected to normalize to 4 nmole dose, the amount used in the behavioral experiments of Flood *et al.* [7].

## RADIOACTIVITY IN BLOOD, LIVER AND BRAIN OF RATS AFTER IP ADMINISTRATION OF COLCHICINE

Exp. No.	nmole Adm	% in Blood*	% in Liver	% in Brain	% of Brain Supernatant		Colchicine Equivalent in Brain <sup>†</sup> (pmoles)
					TCA Insoluble	Vinblastine Precipitable	
[Ring A-4- <sup>3</sup> H]-Colchicine							
1 Hr							
13R	14.8	0.36	0.8	0.0018	5	52	0.15
17R	16.4	0.50	8.5	0.0020	7	56	0.18
3Hr							
1R	1.1	0.22	2.1	<0.01	—	—	—
17R	16.4	0.23	2.6	0.0024	4	40	0.15
12R	17.8	0.16	5.4	0.0062%	4	4	0.04
24 Hr							
13R	14.8	0.13	0.08	0.0030	1	3	<0.02
17R	16.4	0.13	0.02	0.0006	1	<10	<0.02
[Ring C-methoxy- <sup>3</sup> H]Colchicine							
1 Hr							
13 Am-M	16.8	[0.07]	0.4	0.0017	0	11	0.03
17 Am-M	12.1	0.30	0.4	0.0096	1	8	0.12
3 Hr							
21 NEN-M	2.0	—	—	0.014	10 <sup>†</sup>	—	—
13 Am-M	16.8	0.18	1.1	0.0020	5	18	0.06
12 NEN-M	15.5	0.30	5.0	0.0236	3	5	0.20
17 Am-M	12.1	0.33	1.0	0.0071	4	12	0.14
24 Hr							
17 Am-M	12.1	0.47	0.5	0.0128	3	7	0.14
13 Am-M	16.8	0.20	0.1	0.0052	1	3	0.02

\*The blood vol was assumed to be 58 ml/kg rat [8]. For Exp. 1, the Wag-rij rat weighed 207 g, the liver weighed 8 g. For Exp. 12, rats weighed 370 g and had 22 g livers. In Exp. 13, Wistar rats weighed 290 g, livers 15 g. The rats used in Exp. 17 weighed 220 g, livers 9 g, and in Exp. 21, the rat weighed 130 g.

<sup>†</sup>The colchicine equivalent was calculated from the percentage of brain supernatant radioactivity precipitated by  $2.5 \times 10^{-3}$  M vinblastine multiplied by the total radioactivity in the brain. The result was multiplied by 16 and divided by the actual dose administered to normalize to a 16 nmole dose, the approximate average of the dose used in the majority of these experiments. In this experiment, the cortex and subcortex of the perfused brain were each homogenized in 10 vol of 0.9% saline.

<sup>‡</sup>The radioactivity was determined in the homogenates by the same procedure as used in Exps. 19 and 20, Table 2.

Distribution								
Walaszek et al, 1960								
Excretion of <sup>14</sup> C- colchicine in mouse, guinea pig, rat, hamster								
Species	Remarks	No. of Animals or Animal Groups	Dose in mgm/kgm	Urine		Feces		Total Unchanged Colchicine Excreted
				Colchicine	Chloroform-soluble Metabolites	Colchicine	Chloroform-soluble Metabolites	
Mice	Normal	11	1.3	8.03 ± 0.57	2.90 ± 0.46	2.00 ± 0.26	—	10.03 ± 0.4
Mice	Tumor-bearing	8	1.3	2.25 ± 0.13 <sup>(2)</sup>	1.00 ± 0.15 <sup>(2)</sup>	2.63 ± 0.26	—	4.78 ± 0.38
Mice	Irradiated 400 r	5	1.3	8.60 ± 1.0	1.62 ± 0.36	0.99 ± 0.28	—	9.59 ± 0.4
Mice	Irradiated 1250 r	5	1.3	10.2 ± 0.8	4.62 ± 0.67	0.32 ± 0.11	—	10.52 ± 0.7
Guinea Pigs	Normal	13	0.1	2.80 ± 0.70	0.55	1.38	1.12	4.18 ± 1.0
Rats	Normal	14	0.5	3.35 ± 0.46	2.31 ± 0.37	6.30 ± 1.4	1.91 ± 0.25	11.2 ± 1.1
Hamsters	Normal	11	10.0	9.02 ± 0.30	1.98 ± 0.30	1.36 ± 0.24	0.93 ± 0.34	10.4 ± 0.5

(<sup>1</sup>) Data expressed in percent of administered dose at end of 48 hours as mean plus or minus standard error.  
(<sup>2</sup>) *p* = 0.01 when compared to normal.

### Excretion in feces and urine

#### Hunter and Klaassen, 1975

Sprague-Dawley rats  
<sup>3</sup>H-colchicine 0.2 mg/kg, i.v.

Rat: During the first 24 hours, 56% of the dose was excreted in the feces and 11% in the urine.

Three days after dosing, 83% of the total radiolabel had been excreted, with 70% recovered in the feces and 14% in the urine.

Tissue concentrations <sup>a</sup> of tritium 20 minutes after administration <sup>b</sup> of <sup>3</sup>H-colchicine

	Rat	Hamster	Rabbit	Dog
Brain	0.091 ± 0.011 <sup>c</sup>	0.079 ± 0.004	0.661 ± 0.136	1.11 ± 0.14
Muscle	1.15 ± 0.06	0.732 ± 0.050	0.736 ± 0.091	3.02 ± 0.40
Stomach		1.11 ± 0.08	2.94 ± 0.43	5.84 ± 1.05
Intestine		2.16 ± 0.31	3.92 ± 0.54	3.18 ± 0.63
Heart	1.50 ± 0.08	0.863 ± 0.086	2.61 ± 0.42	3.84 ± 0.70
Lung	2.02 ± 0.12	1.10 ± 0.15	4.58 ± 0.48	4.11 ± 0.42
Spleen	2.70 ± 0.22	1.56 ± 0.24	18.0 ± 1.8	6.84 ± 0.37
Kidney	8.81 ± 0.74	6.45 ± 0.89	8.38 ± 1.36	9.51 ± 0.94
Liver	9.63 ± 0.89	3.44 ± 0.15	3.89 ± 0.59	1.90 ± 0.27
Plasma	0.783 ± 0.041	1.18 ± 0.09	2.97 ± 0.53	1.92 ± 0.31
Blood	0.836 ± 0.045	0.670 ± 0.086	69.8 ± 4.5	1523 ± 273
Bile	542 ± 55	198 ± 23.1		

<sup>a</sup> Micrograms per gram or micrograms per milliliter.

<sup>b</sup> 2.0 mg/kg dose was given to each animal.

<sup>c</sup> Each value represents the mean ± S.E. of three to five animals.

Concentration ratios <sup>a</sup> of colchicine in the rat, hamster, rabbit and dog

	Bile/Plasma	Liver/Plasma	Bile/Liver
Rat	737 ±66 <sup>b</sup>	13.0 ±0.6	50.3 ±3.6
Rabbit	18.7 ±1.5	2.15 ±0.09	8.77 ±0.94
Dog	870 ±242	5.12 ±0.41	162 ±31
Hamster	167 ±12	2.99 ±0.23	57.8 ±6.1

<sup>a</sup> Determined 20 minutes after i.v. administration of colchicine (2 mg/kg).

<sup>b</sup> All values represent the mean ± S.E. of 3 to 4 animals.

## Biliary excretion

### Hunter and Klaassen (1975a)

Anesthetized rats, hamsters, rabbits, and dogs with bile duct cannula

Radiolabeled colchicine 2.0 mg/kg, i.v.

Species differences in secretion of colchicine ranged from 16 to 50%.

Rat: Peak biliary excretion rate was achieved within 5 minutes of dosing. During the first 24 hours, 56% of the dose was excreted in the feces and 11% in the urine. Three days after dosing, 83% of the total radiolabel had been excreted, with 70% recovered in the feces and 14% in the urine.

Pharmacokinetic data of colchicine (1 mg/kg) in rat blood, liver and bile, both with and without treated with CsA (20 mg/kg) or proadifen (10 mg/kg)			
Drug treatment	Colchicine (1 mg/kg)		
	Colchicine alone	With CsA	With proadifen
<b>Blood</b>			
AUC (min $\mu\text{g/ml}$ )	14.8 $\pm$ 1.7	24.7 $\pm$ 2.2*	16.2 $\pm$ 2.3
$C_{\text{max}}$ ( $\mu\text{g/ml}$ )	1.3 $\pm$ 0.4	0.9 $\pm$ 0.2	1.0 $\pm$ 0.2
Cl (ml/(kg min))	72.9 $\pm$ 9.9	42.2 $\pm$ 4.3*	67.1 $\pm$ 7.8
MRT (min)	16.0 $\pm$ 3.9	38.7 $\pm$ 3.5*	21.0 $\pm$ 1.6
<b>Liver</b>			
AUC (min $\mu\text{g/ml}$ )	23.1 $\pm$ 6.8	14.1 $\pm$ 1.3	75.9 $\pm$ 15.6*
$C_{\text{max}}$ ( $\mu\text{g/ml}$ )	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	1.7 $\pm$ 0.4*
MRT (min)	47.2 $\pm$ 3.9	45.3 $\pm$ 3.8	51.4 $\pm$ 3.5
<b>Bile</b>			
AUC (min $\mu\text{g/ml}$ )	1756 $\pm$ 441	315 $\pm$ 53*	1716 $\pm$ 94
$C_{\text{max}}$ ( $\mu\text{g/ml}$ )	48.5 $\pm$ 9.1	5.4 $\pm$ 0.8*	43.7 $\pm$ 2.5
MRT (min)	72.5 $\pm$ 4.1	111.9 $\pm$ 6.2*	77.2 $\pm$ 5.0
AUC <sub>liver</sub> /AUC <sub>blood</sub>	1.8 $\pm$ 0.6	0.6 $\pm$ 0.05	5.0 $\pm$ 1.0*
AUC <sub>bile</sub> /AUC <sub>blood</sub>	121.6 $\pm$ 24.7	12.9 $\pm$ 1.8*	115.2 $\pm$ 15.2
Data are expressed as mean $\pm$ S.E. mean ( $n=6$ ). * $p < 0.05$ significantly different from the colchicine alone group.			
<b>Renal Excretion</b>			
<b>Speeg et al., 1992</b>			
Male Sprague-Dawley rats, anesthetized, bile-duct and ureter cannulated Administration of an intravenous bolus (5.5 mg/kg) of colchicine followed by a constant infusion (231 $\mu\text{g/min/kg}$ )  After achieving steady state, serial samples of urine and blood collected  Co-administration evaluated with one of the following: cyclosporine (CsA), cremophor, p-aminohippurate (PAH), ranitidine, tetraethylammonium (TEA) bromide, N-methylnicotinamide (NMN), or probenecid  Additional serial urine and blood samples collected  Renal clearance was calculated: $\text{CLr} = (\text{urine concentration} \times \text{urine volume}) / (\text{plasma concentration})$ .	Renal clearance accounted for ~16% of overall clearance.  $\text{CLr} = 6.92 \pm 0.45 \text{ mL/min}\cdot\text{kg}$ Average urine flow = $63.24 \pm 3.84 \mu\text{L/min}$ GFR was $4.49 \pm 0.13 \text{ mL/min}\cdot\text{kg}$ Average secretory ratio ( $\text{CLr} / \text{GFR}$ ) = $1.482 \pm 0.095$ Data indicates net secretion of colchicine into urine.  Co-administration of organic ions, e.g., TEA and NMN or the OATP inhibitor, probenecid, had no effect on this ratio. With CsA co-administration, the renal clearance of colchicine was reduced to approximately GFR.		
<b>Drug-Food Interaction</b>			
<b>Benmoussa et al 1995</b>			
Rat Effect of non-absorbable fat substitutes [sucrose polyester (SPE) and tricarballylate triester (TCTE)] on the enterohepatic circulation of colchicine	Exp 1: Treatments did not affect plasma levels of colchicine (previous experiment in bile duct-cannulated rats showed that SPE and TCTE, introduced by intragastric tube, have no effect on		

<p>Exp 1: Emulsions of either sunflower oil (SFO), SPE, or TeTE, were introduced into the ligated small intestine and compared to a control group receiving physiological saline. All the groups received colchicine as an intravenous bolus.</p> <p>Exp 2: Colchicine diluted in bile was mixed with saline or emulsions of either SFO, SPE or TCTE, and introduced into the ligated small intestine.</p>	<p>bile flow rate).</p> <p>Exp 2: AUC and Cmax were reduced when the drug was mixed with SPE or TCTE compared to saline. After 150 min, luminal samples had significantly higher (<math>p &lt; 0.034</math>) concentrations of colchicine in both SPE and TCTE groups compared to the saline group.</p> <p>In conclusion, the non-absorbable fat substitutes, SPE and TCTE, did not influence biliary excretion of colchicine but reduced its reabsorption, thus altering its enterohepatic circulation.</p>																																			
<p>Tables from <b>Benmoussa et al (1995)</b>:  Pharmacokinetic Parameters of [<math>^3\text{H}</math>]Colchicine after i.v. administration, with the small intestine filled with saline, or emulsion of SFO, SPE, or TCTE.  The Results are Expressed as Mean <math>\pm</math> SEM (n =6). Statistics: Not Significant For All Groups.</p>																																				
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## 6 General Toxicology

The applicant did not conduct toxicology studies. The published nonclinical literature contains limited information for chronic treatment studies and most toxicology studies are only a few weeks duration. Furthermore, almost all the studies were conducted prior to GLP regulations and lack much of the information now routinely collected such as clinical pathology and histopathology findings. Nevertheless published animal studies and clinical experience indicated similar toxicities with increasing doses. However, as noted by the applicant, due to objectives of determining colchicine's effects and toxicity rather than a safe dose (NOAEL) of colchicine, the studies in animals do not enable an assessment of a safe colchicine dose.

**Animal LD<sub>50</sub> and human lethal dose**

On an acute basis, the LD<sub>50</sub> in rats is approximately 1.7 mg/kg for intravenous administration i.v., which is similar to the LD<sub>50</sub> following i.p. injection. The appearance of clinical effects at lethal doses are delayed, taking 3 to 6 hours to appear after the highest doses (up to 4 mg/kg) and up to 24 hours to appear after lower doses of 0.5 to 2 mg/kg (both routes). The LD<sub>50</sub> is less than 1.0 mg/kg, i.v., in rabbits and 0.9 mg/kg, p.o., in dogs. These values are similar to doses that have been reported to be fatal in human overdose (some fatalities resulted from dose levels between 0.5 to 0.8 mg/kg with 100% fatality at doses greater than 0.8 mg/kg). Similar to that reported in animal studies, several reports of fatal human overdoses of colchicine have noted delayed death (Brvar et al., 2004; Hung, et al., 2005; Milne and Meek, 1998; Putterman et al 1991; and Stapczynski et al 1981). The hamster, a species in which the extent of metabolism appears higher than in other species, apparently is more resistant to the usual toxic effects of colchicine in vivo, as evidenced by survival following single i.p. injections of colchicine as high as 70 mg/kg without adverse effects.

**Table 6: Single and Repeat Dose Toxicity**

Species / Dose of Colchicine	Findings
<b>Ferguson, 1952</b>	
<p><b>Single Dose</b> Rat, iv:0.5 to 4 mg/kg; ip: 4 mg/kg</p> <p>Cat, iv: 0.12 to 1 mg/kg</p> <p><b>Repeated Dose</b> Rat 0.1 mg/kg/day, ip for 5 days, doubled each week for five weeks (final dose was 1.6 mg/kg/day)</p> <p><b>Tolerance assessment:</b> 20 of the rats injected for 3 weeks, last dose 0.4 mg/kg, then given a 2 or 5 day rest and then injected with 4 mg/kg</p>	<p><b>Single Dose</b> <b>Rat:</b> IV LD<sub>50</sub> 1.7 mg/kg Death was delayed between 2 and 13 days in 10 of the rats.</p> <p><b>Cat:</b> IV LD<sub>50</sub> 0.25 mg/kg Death was delayed between between 2 and 12 days in 8 of the cats.</p> <p><b>Repeated Dose</b> <b>Rat:</b> 0.4 mg/kg/day: weight loss, ascites 5% of the animals died 0.8 mg/kg/day: diarrhea additional 4% of animals died 1.6 mg/kg/day: disheveled, bloody staining of the nose, severe diarrhea, hind leg paralysis. 35% of the animals died after 3 injections</p> <p><b>Tolerance assessment:</b> 18 of the 20 rats died indicating that there was no significant tolerance</p>
<b>Hunter and Klaassen (1975b)</b>	
<p>Young rats Colchicine ip single dose of 10 mg/kg</p>	<p>5 and 10 days of age: LD<sub>50</sub> = 0.24 mg/kg 15 days of age: LD<sub>50</sub> = 0.51 mg/kg 25 or 35 days of age: LD<sub>50</sub> = 2.0 mg/kg</p>
<b>Dasheiff and Ramirez (1985)</b>	

Anesthetized rats, 0.25 to 25 µg colchicine into the hippocampus	Dose-dependent destruction of dentate granule cell (DGC) bodies 1 week later. 25 µg resulted in destruction of both dentate granule cells and pyramidal cells.  No behavioral changes were observed.  Comparison to other tubulin-binding drugs indicated that colchicine is not a neurotoxin, rather causing brain damage via a nonspecific inflammatory mechanism.
Rhesus monkeys, 5 to 200 µg of colchicine in each of 4 different sites in the hippocampus (20 to 800 µg total dose)	Less selective and more severe damage than in rats. 5 µg dose in 4 sites produced a large area of cystic necrosis with a surrounding zone of non-selective neuronal death and inflammation.
<b>Goldschmidt and Steward (1989)</b>	
Rat Single dose, injection into dorsal hippocampus 0.7µL of a 9.5 nmol colchicine solution	Massive cell death 2 days after injection of colchicine into the dentate gyrus, with extensive gliosis and appearance in the granule layer of numerous small darkly staining cell fragments and debris.  Since there is very low brain penetration of colchicine, this data is probably not relevant to clinical practice.

## 7 Genetic Toxicology

The published studies indicated that colchicine was negative in most mutagenic assays, but either positive and negative for clastogenic assays. Positive mutagenic effects were reported for the in vitro mouse lymphoma tk assay at doses of 10 to 50 ng/mL, in the in vitro mammalian cell micronucleus assay (0.1 µg/mL), and the in vivo micronucleus in mice (0.35 to 5 mg/kg), hamsters (3 mg/kg), and rats (2.5 to 5 µg/kg). In general, positive chromosomal aberration assays were obtained with higher doses than the doses in studies that had negative results.

The assays conducted with mammalian cells require cellular proliferation (mitosis) as part of the methodology. The study of Honma et al., (2001) determined that the mutagenic effects in the in vitro mouse lymphoma tk assay were due to loss of a functional tk allele generated by the loss of the entire chromosome 11 where the tk gene resides. There were no mutants with structural changes such as deletions or translocations involving chromosome 11. The mutations observed in the assay arose through mitotic nondisjunction without structural DNA changes.

In the published clastogenic assays, cells with micronuclei were counted, but not examined at the chromosome level for signs of clastogenicity. Jie and Jia (2001) examined the micronuclei and found they were composed mainly of whole chromosomes. These findings are consistent with colchicine's well characterized induction of aneuploidy. Micronuclei can arise from acentric fragments induced by substances causing chromosomal breakage (clastogens), as well as from whole lagging chromosomes induced by those causing aneuploidy. Therefore, the positive results in

assays are not due to mutagenic or clastogenic mechanism, but results from colchicine's mechanism of inducing aneuploidy.

**Table 7: Genotoxicity Studies**

Species/Strain	Method	Genotoxic Effects
<b>Ames Assay: CCRIS Toxnet</b> citing "Short-term test program sponsored by the division of cancer biology, NCI, p. Y85		
Salmonella typhimurium TA98 TA100 TA1535 TA1537 TA1538	Dose: 100-1000 µg/plate Vehicle: water Metabolic Activation, two types studied: ±rat liver S-9, aroclor 1254 and ±hamster liver S-9, aroclor 1254 Standard plate methodology	Negative
<b>Mouse Lymphoma Assay: CCRIS Toxnet</b> citing "Short-term test program sponsored by the division of cancer biology, NCI, Ms. Ellen Zaika, Assistant Project Officer, page Y85		
Mouse, lymphoma cells, L5178Y (TK <sup>+</sup> /TK <sup>-</sup> )	Dose: 0.01-0.06 µg/mL no S-9 Dose: 0.05-0.1 µg/mL with S-9 Vehicle: water Metabolic Activation: ±rat liver S-9, aroclor 1254 Suspension plate methodology	Negative
<b>Mouse Lymphoma Assay: Honma et al 2001</b>		
Mouse, lymphoma cells, L5178Y (TK <sup>+</sup> /TK <sup>-</sup> )	Dose: 25 ng/mL without S-9 Vehicle: saline Metabolic Activation: ±rat liver S-9, aroclor 1254 Microwell methodology	Positive
<b>Mouse Lymphoma Assay: Yuan et al 2003</b>		
Mouse, lymphoma cells, L5178Y (TK <sup>+</sup> /TK <sup>-</sup> )	Dose: 10-50 mg/mL without S-9 Vehicle: not reported 24 hr treatment	Positive
<b>In Vitro Micronucleus: Matushima et al 1999</b>		
CHL/IU cells	Dose: 0.012 to 12 µg/mL Vehicle: saline No metabolic activation 48 hr continuous treatment	Positive
<b>In Vitro Micronucleus: Fellow and O'Donovan 2007</b>		
Mouse lymphoma cells, L5178Y, (TK <sup>+</sup> /TK <sup>-</sup> ) / trifluorothymidine	Dose: 0, 0.0125, 0.025, 0.031, 0.044, 0.05, 0.063, 0.075, 0.0875 µmol/L Vehicle: distilled water Metabolic Activation: none 24 hr continuous treatment;  As above with cytochalasin B added at beginning of 24 hr recovery period	Negative  Positive
<b>In Vivo Micronucleus: De Boek et al 2005</b>		
CD-1 Mouse	Dose: 3, 6, or 12 mg/kg, single dose, oral route,	Positive

Examination of blood erythrocytes		
<b>In Vivo Micronucleus: Igarashi et al 2007</b>		
Mouse strain ddY, male	Dose: 0.375, 0.75, 1.5 mg/kg, i.v. Vehicle: saline Colchicine administered 1 day before or 1 day after partial hepatectomy; hepatocytes sampled 6 days after dosing	Positive for micronucleated, binucleated, and multinucleated cells after partial hepatectomy
<b>In Vivo Micronucleus: Cohen et al 1997</b>		
Human peripheral lymphocytes	Peripheral lymphocytes from patients with Familial Mediterranean Fever (FMF): 14 male and 8 female under treatment from 1 week to 5 years  Controls: 4 male and 6 female  Stimulated to divide with phytohemagglutinin  Cultures were incubated for 72 hours with 0.01 to 100 µg/mL of colchicine	No difference in mitotic rate, percent tetraploidy and chromosome breakage, compared to controls.  Incubating these cultures for 72 hours with colchicine increased mitotic rate but not tetraploidy or chromosome damage.  The increased mitotic rate with colchicine incubation was likely an artifact of the assay, in which colchicine arrested mitosis as cell entered that phase (providing an accumulated mitotic count), but non-colchicine treatment enabled the cells to continue to divide and thus only those cells dividing at 72 hr timepoint were counted.

## 8 Carcinogenicity

There were no adequate studies of colchicine's carcinogenicity potential. The study cited by the sponsor (Paget et al, 1960), although negative for colchicine induced tumors, was too short in duration and lacked sufficient numbers of animals to make an assessment for a drug marketing application. Due to the long history of clinical experience and age of the patient population, carcinogenicity studies were not requested for this application.

**Table 8: Effects on Carcinogenicity**

Method	Findings
<b>Paget et al (1960)</b>	
Wistar rats weaned at the commencement of the experiment. 10/sex/group Group I: colchicine, 0.5 mg/kg 2X/week for 42 weeks, initially SC in saline. but due to local necrosis the vehicle was changed to arachis oil  Group II: control, saline, then switched to arachis oil after 4 weeks. Animals were followed for 93 weeks then sacrificed for histopathological	In the colchicine treated group, by week 52 there were 8 deaths and by week 78 there were 9 deaths. There was no mention of the number of deaths in the control group, nor of the number of animals of each sex that died.  The number of survivors at week 93 were 5 in the colchicine group and the number of control survivors was not mentioned. The sex of the animals was not provided.  Tumors in survivors at week 93 were as follows: control: 4 adenomata of thyroid, 1 leiomyosarcoma of the vagina, and 1 sarcoma at injection site. colchicine: 1 fibroadenoma of mammary gland

examination of major organs	<p>The sex of the animals with tumors was not provided.</p> <p>Nontumor findings: liver and stomach mucosa appears more variable in size than normal (the treatment group or groups with this finding were not identified)</p>
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## 9 Reproductive and Developmental Toxicology

Published nonclinical studies indicated that colchicine has adverse effects on sperm development (fertility), early embryonic development and implantation, organogenesis (teratology), and late-stage embryonic development. These findings are consistent with its pharmacodynamic effect as an inhibitor of microtubule formation which results in the disruption of cytoskeletal functions, cell movement, and cell division. The effects are species and dose dependent, and the timing of exposure is critical for the effects on embryonic development. In males, colchicine interfered with seminiferous tubule fluid secretion, testosterone production/release from rat Leydig cells, and disrupted microtubules in Sertoli cells in the epididymides, resulting in abnormal sperm production. In females, colchicine administration can result in eggs with Y chromosomes, and interfere with sperm penetration, the second meiotic division, and normal cleavage, and produce triploid and mosaic embryos. Colchicine in animal studies was found to be embryo-lethal early in development, is associated with the production of skeletal abnormalities during organogenesis, and causes slow embryofetal development. Published studies indicate that colchicine-mediated microtubular disruption inhibits the secretion of various hormones necessary for reproduction and maintenance of pregnancy. Colchicine also reduced milk yield and altered milk composition (reduced fat content).

The applicant stated that the animal studies are not predictive of human fertility. Nonclinical studies were conducted to demonstrate an effect of colchicine, to define mechanisms involved in different stages of development and identify colchicine's potential toxicities, but not to determine a safe level of colchicine use. In contrast to these detrimental effects, the therapeutic use of relatively very low amounts of colchicine for the treatment of gout or familial Mediterranean fever was found to be compatible with reproduction, pregnancy, lactation and growth in patients with familial Mediterranean fever.

The applicant referred to epidemiological data from patients on colchicine when asked to provide information concerning placental transfer and postnatal development. They also provided clinical information concerning colchicine transfer into breast milk. These studies were not reviewed here, refer to the clinical review. The information in the following tables of nonclinical fertility and pregnancy studies of colchicine were cited by the applicant or included within review articles cited by the applicant.

**Table 9: Nonclinical Fertility and Early Embryonic Development**

<b>Method</b>	<b>Findings</b>
<b>Correa et al (2002)</b>	
Adult male Swiss Webster mice, Colchicine in PBS was intratesticularly administered (25 µl) at 5.3 or 117.6 µg/g testis Contralateral testis served as control  Histology of tissue at 6 h after treatment Rat was the positive control for the mouse study in which colchicine in PBS was intratesticularly administered (50 µl) 5.3 µg/g rat testis  In another earlier rat study, colchicine was administered to testis at 5.6 µg/g	Low dose of colchicine in mice caused enlarged lumen, but no sloughing of germ cells or vacuolation were observed. High dose caused massive sloughing of germ cells, germ cells (spermatids and spermatozoa) with attached apical epithelia completely detached from the basal epithelium.  Rat: 5.3 µg/g testis produced sloughing of germ cells.  Rat: Damage to the seminiferous epithelium and microtubule cytoskeleton in the rat.
<b>Wyrobek and Bruce, (1975)</b> <b>Bruce and Heddle (1979)</b>	
Mouse, (C57BL x C3H) 0.6 and 0.75 mg/kg, i.p. colchicine over 5 days, sacrificed at 1, 4, and 10 weeks postdose	Colchicine treatment increased the fraction of abnormally shaped sperm at weeks 1 and 4. (used to develop a sperm abnormality assay for screening various compounds)
<b>Rat</b>	
<b>Piko and Bomsel-Helmreich (1960)</b>	
Rat: Wistar CF, Long Evans, and Sherman strains 0.25 to 0.5mg/kg, i.p.colchicine at 2 to 2.5 hours after mating in the rat coupled with hyperthermia (heated box) to aid in inducing polyspermy	Colchicine treatment resulted in diploid, triploid and mosaic embryos and androgenetic eggs (formed by the combination of two male pronuclei). The frequency of abnormalities depended on time of application in relationship to mating.  Previous results demonstrated that colchicine suppressed second polar body and produced eggs with single pronucleus (presumably male) and with subnuclei in place of female pronuclei.
<b>Allard et al 1993</b>	
Rat, Charles River CD intratesticular injection of colchicine, 0.004-40 µg/testis	<ul style="list-style-type: none"> <li>• Dose-related decrease in seminiferous tubule fluid secretion and completely blocked secretion at 40 µg/testis.</li> <li>• A dose-related decrease in testis weight was observed at 2 and 8 weeks after dosing.</li> <li>• Time dependent increase in incidence of sloughing over 1 to 16 hours with stages IX-XIV most sensitive.</li> <li>• Tubulin immunostaining in Sertoli cells was altered preferentially in stage VII-VIII seminiferous tubules.</li> </ul>
<b>Rabbit</b>	
<b>Barsoum (1955)</b>	
Rabbit 1.5 or 3 mg/kg, SC colchicine, twice weekly for 15 weeks	Testes weight reduced, spermatogenesis arrested in colchicine treated rabbits compared to controls.
<b>Adams et al. (1961)</b>	
Rabbit	No interference with ovulation and fertilization at these doses.

<p>Single injections of Colcemid (demecolcine, related to colchicine, less toxic) ranging from 0.3 to 8.0 mg/kg</p>	<p>Treatment with low doses of Colcemid on alternate days for 3 weeks did not interfere with mating and conception. Administration of 2-5 mg/kg caused arrest of ovum cleavage within a few hours of the injection. The effect on the histology of the blastocysts (at 5.5, 6.5, and 6.67 days of age) was related both to the time of administration and the dose level. In long-term experiments, 1 mg/kg or less, given on day 1 or 2, had no visible effect at 6 days, but if given on day 4 or 5 embryonic disc development was inhibited. The injection of 2 mg/kg on day 5, 36 hours before autopsy, caused small or collapsed blastocysts with irregular and degenerating discs. The trophoblasts had signs of damage. Rabbits given 0-5 mg/kg on alternate days for 3 weeks before mating carried their litters to term. At birth the young were rather small but there were no obvious malformations. However the litters did not survive which was proposed to be due to lactational insufficiency.</p>
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**Table 10: Nonclinical Pregnancy**

Method	Findings										
<b>Mouse</b>											
<b>Didcock et al. (1956)</b>											
<p>Mouse Colchicine at 1, 1.5 or 2 mg/kg, SC, on day 10 or 11</p> <p>Other compounds related to colchicine, either natural or chemically modified, were also studied</p>	<p>Produced interruption of pregnancy.</p> <table border="1" data-bbox="532 972 1263 1199"> <thead> <tr> <th data-bbox="540 982 667 1108">Animals</th> <th data-bbox="675 982 821 1108">Duration of Pregnancy at Time of Injection (Days)</th> <th data-bbox="829 982 976 1108">Dose of Colchicine (mg./kg.)</th> <th data-bbox="984 982 1130 1108">Interruption of Pregnancy*</th> <th data-bbox="1138 982 1255 1108">Maternal Mortality</th> </tr> </thead> <tbody> <tr> <td data-bbox="540 1119 667 1199">Mice ..</td> <td data-bbox="675 1119 821 1199">11 10 11</td> <td data-bbox="829 1119 976 1199">2 1-5 1</td> <td data-bbox="984 1119 1130 1199">4/4 2/5 1/5</td> <td data-bbox="1138 1119 1255 1199">1/4 1/5 0/5</td> </tr> </tbody> </table>	Animals	Duration of Pregnancy at Time of Injection (Days)	Dose of Colchicine (mg./kg.)	Interruption of Pregnancy*	Maternal Mortality	Mice ..	11 10 11	2 1-5 1	4/4 2/5 1/5	1/4 1/5 0/5
Animals	Duration of Pregnancy at Time of Injection (Days)	Dose of Colchicine (mg./kg.)	Interruption of Pregnancy*	Maternal Mortality							
Mice ..	11 10 11	2 1-5 1	4/4 2/5 1/5	1/4 1/5 0/5							
<b>Bellies, 1972</b>											
<p>Comparison of colchicine in pregnant vs nonpregnant CD-1 mice</p> <p>Day 13 of pregnancy administration of doses, day 17 cesarean section and necropsy Nonpregnant mice sacrificed after 7 days</p>	<p>Pregnant mice were more sensitive to the toxicity of colchicine than non-pregnant mice based on LD<sub>50</sub> values:</p> <p>Nonpregnant IV LD<sub>50</sub> = 4.13 mg/kg Pregnant IV LD<sub>50</sub> = 1.54 mg/kg</p>										
<b>Rat</b>											
<b>Petit and Isaacson (1976)</b>											
<p>Pregnant rats injected with 0.4 mg/kg colchicine on embryonic days 18, 19, and 20</p>	<p>Offspring had reduced size of cortical and hippocampal structures and pyknotic nuclei within these regions (or in areas that are known to send axons to these regions) when examined at birth. Behavioral and learning deficits were observed as animals matured.</p>										
<b>Thiersch (1958)</b>											
<p>Rat, Long Evans strain ip injection on 2 consecutive days at various times during pregnancy, 1-7 mg/kg of chemically modified</p>	<p>Both TC and MC acted on fetuses within hours and induced general edema, ascites and death. In pregnant rats up to midterm, and frequently also at 11 th and 12th days of gestation, the fetus would be resorbed with</p>										

colchicine: N-desacetyl-methyl-colchicine (MC), (related to colchicine, 7-fold less toxic based on LD <sub>100</sub> ) N-Desacetyl Thio Colchicine (TC), (25-100-fold less toxic than colchicine based on LD <sub>50</sub> ) fetuses examined on d 21 of gestation	placentas surviving. In rats pregnant in the second half of their gestation period, the fetuses died but remained often as a macerated mass Organs of dams were normal, except bone marrows and intestinal epithelium of rats treated on days 15 and 16. or 18 and 19 and sacrificed within 48 hours in which theree was 30% depletion of cellular contents of marrow and increased number of mitosis in the crypts of intestinal mucosa.
<b>Tuchmann-Duplessis and Mercier-Parot (1958)</b>	
Rat, Wistar strain, colcemide 0.2 or 0.5 mg,SC, gestation days 7, 8 and 9	Embryocidal effects 0.2 mg: 40 % resorptions, no malformations in viable fetuses 0.5 mg: not compatible with gestation
<b>Rabbit</b>	
<b>Chang (1944)</b>	
Rabbit Sperm suspended in 0.1% colchicine solution and artificially inseminated female rabbits	33 young resulted: 1 - open fontanelle and very small philtrum 1 - one was otocephalic 1 - microcephaly with enlarged eyes.  Doses of similar origin were artificially inseminated and resulted in 425 normal young.
<b>Didcock et al. (1956)</b>	
Rabbit 1.5 to 18 mg/kg, oral and SC on various single days during organogenesis	Produced interruption of pregnancy due to fetal or maternal death. 1 rabbit had hemorrhagic reabsorption and died within a day, the other died on day 2 post injection with no effect on fetus.
<b>Morris et al. (1967)</b>	
Rabbit, New Zealand white 0.1 to 5.0 mg/kg	High dosages after the 9th day were highly lethal to fetuses, death within 2-4 hrs 5 mg/kg, 50% of dams died from toxicity Teratogenic effects: small incidence of gastroschisis failure of neural tube closure
<b>Monkey</b>	
<b>Morris et al. (1967)</b>	
Monkey 1 to 2 mg/kg single-dose colchicine on days 24, 45, 66, and 84 of pregnancy	Four normal fetuses, no abnormalities observed

## 11 Integrated Summary and Safety Evaluation

With a long clinical history for the use of colchicine, the applicant submitted published nonclinical literature to support this 505(b)(2) NDA. No new nonclinical studies were conducted or requested.

### Pharmacology

Colchicine binds to the intracellular protein tubulin, preventing its alpha and beta forms from polymerizing into microtubules. This disruption of the microtubular network results in impaired protein assembly in the Golgi apparatus, decreased endocytosis and exocytosis, altered cell shape, depressed cellular motility and arrest of mitosis. Colchicine also interferes with the formation of the inflammasome, a newly appreciated and identified cellular structure involved in the production of inflammatory-related cytokines. In addition, colchicine also prevents neutrophil migration from the vasculature into tissue by preventing the expression of cell surface adhesion-related molecules E- and L-selectins.

### General toxicology

Historical information provided by the applicant from published animal studies and human use indicates similar toxicities with increasing doses. However, the published nonclinical literature contains inadequate long-term toxicology studies. Almost all the studies were conducted prior to GLP regulations and lack much of the information now routinely assessed, such as clinical pathology and histopathology findings. In general, the acute toxic signs in animals (rats, dogs, rabbits, cats) with short-term colchicine administration are gastrointestinal tract-related and include emesis, distended intestines, diarrhea (bloody in more severe cases), lack of appetite, and lethargy. With increasing doses these signs become more severe, and there is a loss of body tone, abnormal gait and hindlimb paralysis and wasting atrophy, ascites and eventually death. For comparison, in humans, at doses just above the narrow therapeutic range, colchicine can produce gastrointestinal disorders, profound muscle weakness, respiratory insufficiency, and peripheral neuropathy.

Colchicine has a very narrow therapeutic window, with human deaths reported at doses not much greater than therapeutic doses. Comparing human lethality with the limited nonclinical data at non-lethal doses indicates that human deaths have been reported at doses lower than those that affect rodents, implying that NOAEL determinations may not provide a useful margin of safety. Furthermore, a direct NOAEL comparison could not be conducted, since for the most part, nonclinical studies were not conducted with the goal of identifying a NOAEL. Rather, the studies were conducted to identify toxicities or underlying biological mechanisms of colchicine action.

### Genetic Toxicology

Published studies reported colchicine was not mutagenic in the majority of Ames tests, but was positive in the *in vitro* mouse lymphoma thymidine kinase (TK) assay, and positive in most clastogenic tests (*in vitro* mammalian cell micronucleus assay and *in vivo* in mice, hamsters, and rats). In retrospect, these were probably false positives, a result of the cellular proliferation essential for these assays. The study of Honma et al (2001) determined that the mutagenic effects in the mouse lymphoma TK assay were due to loss of a functional *tk* allele generated by the loss of chromosome 11 on which the *tk* gene is located. There were no mutants with structural changes such as deletions or translocations involving chromosome 11. The mutations described in this assay

arose through mitotic nondisjunction without structural DNA changes. In the published clastogenicity assays, cells with micronuclei were counted, but the chromosomes were not examined for signs of clastogenicity. The study of Jie and Jia (2001) examined the micronuclei and found they were composed mainly of whole chromosomes. These findings are consistent with colchicine's well characterized induction of aneuploidy. Micronuclei can arise from acentric fragments induced by substances causing chromosomal breakage (clastogens) as well as from whole lagging chromosomes induced by those causing aneuploidy. Therefore the positive results in assays are not due to mutagenic or clastogenic mechanism, but results from colchicine's mechanism of inducing aneuploidy.

### Carcinogenicity

There are no adequate studies of colchicine's carcinogenicity potential. Due to the long history of clinical experience and age of the patient population, carcinogenicity studies were not requested for this application.

A recent study by Kuo et al (2012), examined the relative risk of cancer from data in a National Health Insurance database of Taiwan between 2000 and 2008 for subjects  $\geq 20$  years of age and with no previous history of malignancy. Gout was associated with increased risk of prostate cancer in males, however there was not a subgroup analysis of patients on colchicine therapy, so there is still no data specifically addressing colchicine and cancer risk.

### Reproduction and Developmental Toxicology

Colchicine disruption of microtubule formation results in reproductive and developmental toxicity by cells involved in meiosis and mitosis. The effects are species and dose dependent, with the timing of exposure also critical for the effects on embryonic development. In general, published nonclinical studies indicated adverse effects on sperm development and fertility, early embryonic development and implantation, organogenesis (teratology), and late-stage embryonic development. In males, colchicine interfered with seminiferous tubule fluid secretion, testosterone production/release from rat Leydig cells, and disrupted microtubules in Sertoli cells in the epididymides, resulting in abnormal sperm production. In females, colchicine administration resulted in eggs with Y chromosomes; and interfered with sperm penetration, the second meiotic division, and normal cleavage; and produced triploid and mosaic embryos. Published nonclinical studies demonstrated that colchicine was embryo-lethal early in development, was associated with the production of skeletal abnormalities during organogenesis, and caused slow embryofetal development. Published studies also indicated that colchicine-mediated microtubular disruption inhibited the secretion of various hormones. It also reduced milk yield and altered milk composition (reduced fat content).

Though nonclinical literature indicates that colchicine has detrimental effects on reproduction and development in animals, clinical epidemiology studies in patients with

familial Mediterranean fever have generally not shown that colchicine therapy during pregnancy has negative effects on fetal growth and development, postnatal growth and development, or on the second generation of children of these patients. At the recommended doses, colchicine appears to be compatible with pregnancy and breast feeding. It is not known if a similar degree of safety is evident in patients taking colchicine for gout, for which the therapeutic dose is usually less than for familial Mediterranean fever. However the patient population with gout is typically beyond the child-bearing age (refer to the clinical review by Dr. Keith Hull).

## **Conclusion**

Based solely on animal studies, there is no basis for determining colchicine is safe. The published studies were not conducted to determine a NOAEL, and the data is generally not amenable to a hazard analysis. However, colchicine in its less pure, plant extract, form has been used medicinally for thousands of years and in a somewhat purer form was FDA-approved in 1961 based mostly on clinical experience. The available animal data since then supports the known clinical safety profile.

For topics supportive of labeling for colchicine's safe use, published non-clinical studies support that colchicine is not genotoxic, neither mutagenic nor clastogenic. Previous clastogenic findings were likely artifacts induced by colchicine's aneuristic properties. There is no data concerning the carcinogenic potential of colchicine, but for the proposed patient population and the given the long history of use, coupled with an absence of genotoxicity, there appears to be minimal human carcinogenic risk. Based on reproductive and developmental animal studies, colchicine is gamete and embryofetal toxic and should not be used by men or woman during the period of conceiving and by women during pregnancy and nursing. However, at the doses and exposures used therapeutically, there are no known concerns with colchicine use in terms of human reproduction and development.

In summary, colchicine may be approved from a nonclinical pharmacological toxicological perspective. Findings from nonclinical studies paralleled the clinical experience of this drug and supported the clinical safety profile, but never defined a safe dose based on current experimental standards.

## Appendix/Attachments

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/s/  
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LAWRENCE S LESHIN  
07/01/2013

MARCIE L WOOD  
07/01/2013

## PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

**NDA/BLA Number: 204820**     **Applicant: Hikma  
Pharmaceuticals, LLC**

**Stamp Date: Oct 5 2012**

**Drug Name: colchicine**     **NDA/BLA Type: 505(b)(2)**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		The PharmTox information is summarized in Module 2 as written and tabulated summaries. Module 4 only contains the reference publications
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Minimal presentation of studies is referenced in Module 4 are presented in Module 2. There is sufficient information to support labeling. Carcinogenicity is not presented since there are no adequate publications, however see comments at the end of this filing review.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).		X	Relies on literature publications in which purity and formulations are unknown and different from the to-be-marketed product. The marketed drug substance is probably purer than the drug substance used in the published pharmacology and toxicology studies, at least there is quantification of its degree of purity in contrast to published studies in which there is no purity information.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?		X	The published studies were not conducted as GLP studies. The majority of studies were conducted prior to the existence of GLP regulations in the late 1970's.

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement  
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR  
NDA/BLA or Supplement**

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			There was no presubmission discussion with Hikma Pharmaceuticals. See comments at the end of this filing review.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	The nonclinical aspects of the label are written similar to that of previously approved Colcrys product (NDA 22-352), also based on the same literature. Exposure margins were not able to be calculated.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		PharmTox response will depend on the Product Quality Reviewer's evaluation, (initially it appears adequate, see impurity comments at the end of this filing review).
11	Has the applicant addressed any abuse potential issues in the submission?		X	Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? \_\_YES\_\_**

**If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.**

**Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.**

1. The following sections of the Module 2 Summaries were indicated as “Not Applicable.” Provide information for your product as it relates to drug safety to address these topics. If there is no information available, indicate that. Also provide justification why carcinogenicity studies were not included in your application or not necessary.

Placental Transfer Studies  
Excretion in Milk  
Postnatal Development  
Carcinogenicity

2. Many of the references cited in Module 2 are missing from the publications provided in Module 4. Provide those missing publications.

Reviewing Pharmacologist

Date

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Team Leader/Supervisor

Date

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/s/  
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LAWRENCE S LESHIN  
11/15/2012

TIMOTHY W ROBISON  
11/15/2012  
I concur